



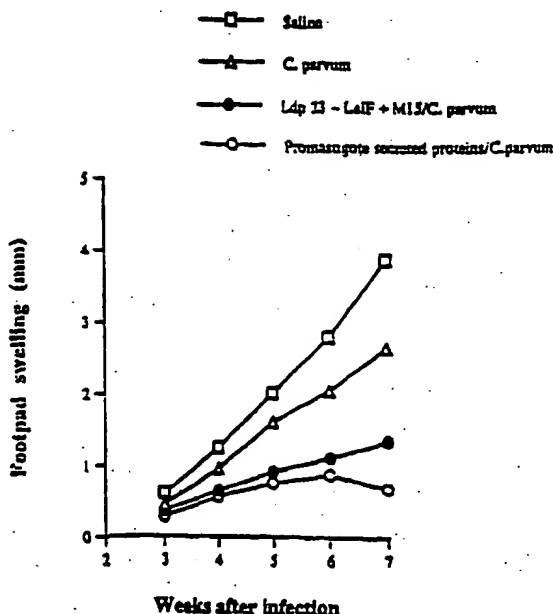
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(54) Title: **LEISHMANIA ANTIGENS FOR USE IN THE THERAPY AND DIAGNOSIS OF LEISHMANIASIS**

(57) Abstract

Compositions and methods for preventing, treating and detecting leishmaniasis and stimulating immune responses in patients are disclosed. The compounds provided include polypeptides that contain at least an immunogenic portion of one or more *Leishmania* antigens, or a variant thereof. Vaccines and pharmaceutical compositions comprising such polypeptides, or DNA molecules encoding such polypeptides, are also provided and may be used, for example, for the prevention and therapy of leishmaniasis, as well as for the detection of *Leishmania* infection.

Protection against infection with *L. major* in BALB/c mice immunized leishmanial antigens.

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LEISHMANIA ANTIGENS FOR USE IN THE THERAPY AND
DIAGNOSIS OF LEISHMANIASIS

REFERENCE TO RELATED APPLICATIONS

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TECHNICAL FIELD

The present invention relates generally to compositions and methods for preventing, treating and detecting leishmaniasis, and for stimulating immune responses in patients. The invention is more particularly related to polypeptides comprising an immunogenic portion of a *Leishmania* antigen or a variant thereof, and to vaccines and pharmaceutical compositions comprising one or more such polypeptides. The vaccines and pharmaceutical compositions may be used, for example, for the prevention and therapy of leishmaniasis, as well as for the detection of *Leishmania* infection.

15 BACKGROUND OF THE INVENTION

Leishmania organisms are intracellular protozoan parasites of macrophages that cause a wide range of clinical diseases in humans and domestic animals, primarily dogs. In some infections, the parasite may lie dormant for many years. In other cases, the host may develop one of a variety of forms of leishmaniasis. For example, the disease may be asymptomatic or may be manifested as subclinical visceral leishmaniasis, which is characterized by mild symptoms of malaise, diarrhea and intermittent hepatomegaly. Patients with subclinical or asymptomatic disease usually have low antibody titers, making the disease difficult to detect with standard techniques. Alternatively, leishmaniasis may be manifested as a cutaneous disease, which is a severe medical problem but is generally self-limiting, or as a highly destructive mucosal disease, which is not self-limiting. Finally, and most seriously, the disease may be manifested as an acute visceral infection involving the spleen, liver and lymph nodes, which, untreated, is generally a fatal disease. Symptoms of acute visceral leishmaniasis include hepatosplenomegaly, fever, leukopenia, anemia and hypergammaglobulinemia.

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Leishmaniasis is a serious problem in much of the world, including Brazil, China, East Africa, India and areas of the Middle East. The disease is also endemic in the Mediterranean region, including southern France, Italy, Greece, Spain, Portugal and North Africa. The number of cases of leishmaniasis has increased dramatically in the last 20 years, and millions of cases of this disease now exist worldwide. About 2 million new cases are diagnosed each year, 25% of which are visceral leishmaniasis. There are, however, no vaccines or effective treatments currently available.

Accurate diagnosis of leishmaniasis is frequently difficult to achieve. There are 20 species of *Leishmania* that infect humans, including *L. donovani*, *L. chagasi*, *L. infantum*, *L. major*, *L. amazonensis*, *L. braziliensis*, *L. panamensis*, *L. mexicana*, *L. tropica*, and *L. guyanensis*, and there are no distinctive signs or symptoms that unambiguously indicate the presence of *Leishmania* infection. Parasite detection methods have been used, but such methods are neither sensitive nor clinically practical. Current skin tests typically use whole or lysed parasites. Such tests are generally insensitive, irreproducible and prone to cross-reaction with a variety of other diseases. In addition, the preparations employed in such tests are often unstable. Thus, there is a need for improved methods for the detection of *Leishmania* infection.

Current experimental vaccines consisting of whole organisms have not proven effective in humans. Accordingly, there remains a need in the art for vaccines to prevent leishmaniasis in humans and dogs, and for improved therapeutic compositions for the treatment of leishmaniasis.

SUMMARY OF THE INVENTION

Briefly stated, the present invention provides compositions and methods for preventing, treating and detecting leishmaniasis, as well as for stimulating immune responses in patients. In one aspect, polypeptides are provided which comprise at least an immunogenic portion of a *Leishmania* antigen, or a variant of such an antigen that differs only in conservative substitutions and/or modifications. In specific embodiments of the invention, the *Leishmania* antigen comprises an amino acid

sequence selected from the group consisting of SEQ ID Nos: 2, 4, 20, 22, 24, 26, 36-38, 41, 50-53 and 82. DNA sequences encoding the above polypeptides, recombinant expression vectors comprising these DNA sequences and host cells transformed or transfected with such expression vectors are also provided.

5 In related aspects, the present invention provides pharmaceutical compositions which comprise one or more of the polypeptides described herein, or a DNA molecule encoding such polypeptides, and a physiologically acceptable carrier. Vaccines which comprise one or more such polypeptides or DNA molecules, together with a non-specific immune response enhancer are also provided. In specific
10 embodiments of these aspects, the *Leishmania* antigen has an amino acid sequence selected from the group consisting of SEQ ID Nos: 2, 4, 20, 22, 24, 26, 36-38, 41, 50-53 and 82.

In still further related embodiments, the pharmaceutical compositions and vaccines comprise at least two different polypeptides, each polypeptide comprising
15 an immunogenic portion of a *Leishmania* antigen having an amino acid sequence selected from the group consisting of sequences recited in SEQ ID Nos: 2, 4, 6, 8, 10, 20, 22, 24, 26, 36-38, 41, 50-53, 82, and variants thereof that differ only in conservative substitutions and/or modifications. In other embodiments, the inventive pharmaceutical compositions comprise one or more of the inventive polypeptides in combination with a
20 known *Leishmania* antigen.

In yet other related embodiments, the pharmaceutical compositions and vaccines comprise soluble *Leishmania* antigens.

In another aspect, the present invention provides methods for inducing protective immunity against leishmaniasis in a patient, comprising administering to a
25 patient a pharmaceutical composition or vaccine as described above.

In further aspects, methods and diagnostic kits are provided for detecting *Leishmania* infection in a patient. The methods comprise: (a) contacting dermal cells of a patient with a pharmaceutical composition as described above; and (b) detecting an immune response on the patient's skin, therefrom detecting *Leishmania* infection in the
30 patient. The diagnostic kits comprise: (a) a pharmaceutical composition as described

above; and (b) an apparatus sufficient to contact the pharmaceutical composition with the dermal cells of a patient.

In further aspects, the present invention provides methods for stimulating a cellular and/or humoral immune response in a patient, comprising administering to a patient a pharmaceutical composition or vaccine as described above.

In a related aspect, methods are provided for treating a patient afflicted with a disease responsive to IL-12 stimulation, comprising administering to a patient a pharmaceutical composition or vaccine as described above.

These and other aspects of the present invention will become apparent upon reference to the following detailed description and attached drawings. All references disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the stimulation of proliferation of T-cells obtained from *L. donovani*-immunized BALB/c mice (represented by stimulation index) by *L. donovani*-infected macrophages after incubation for 24, 48 and 72 hours.

Figure 2 illustrates representative HPLC profiles of peptides isolated from MHC class II molecules of P388D1 macrophages. Panel A shows peptides isolated from uninfected macrophages and panel B shows peptides isolated from *L. donovani* infected macrophages. The arrows in panel B indicate peptide peaks present only in the infected macrophage preparation.

Figure 3 illustrates the expression and purification of the *Leishmania* antigen Ldp23 as a recombinant fusion protein. Panel A shows a Coomassie blue-stained SDS-PAGE gel of lysed *E. coli* without (lane 1) and with (lane 2) IPTG induction of Ldp23 expression. Arrow indicates the recombinant fusion protein. Panel B shows the fusion protein following excision from a preparative SDS-PAGE gel, electroelution, dialysis against PBS and analytical SDS-PAGE.

Figure 4 presents a Northern blot analysis of total RNA prepared from *L. donovani*, *L. major*, *L. amazonensis* and *L. pifanoi* with a ³²P labeled Ldp23 gene. 1, 2

and 3 refer to RNA obtained from promastigotes at the logarithmic growth phase, promastigotes at the stationary growth phase and amastigote forms, respectively.

Figure 5 shows a Western blot analysis of *L. donovani* promastigote antigens incubated with pre-immune rabbit serum (lane A) or with anti-Ldp23 rabbit antiserum (lane B).

Figure 6 illustrates the surface expression of Ldp23 on live *L. donovani* promastigotes. The dotted line shows the indirect immunofluorescence performed using pre-immune mouse serum and the solid line shows the result obtained with mouse anti-GST-Ldp23 antiserum. Fluorescence intensity was analyzed by FACScan.

Figure 7 shows the stimulation of *Leishmania*-specific T-cell proliferation by Ldp23. The results are presented as relative cell number as a function of fluorescence intensity. T-cells (10^5 /well) were purified from lymph nodes of BALB/c mice immunized in the foot pad with *L. donovani* promastigotes in CFA and were cultured with various concentrations of the purified recombinant Ldp23 in the presence of 2×10^5 Mitomycin C-treated normal BALB/c spleen mononuclear cells. Proliferation of T-cells was measured at 27 hours of culture. Values are expressed as cpm and represent the mean of [3 H]TdR incorporation of triplicate cultures.

Figure 8 illustrates Ldp23-induced cytokine production by lymph node cells of BALB/c mice. Cultures were incubated with varying amounts of Ldp23 or *Leishmania* lysate, presented as μ g/mL, and were assayed by ELISA for the production of interferon- γ (panel A) or interleukin-4 (panel B), both of which are shown as ng/mL.

Figure 9 shows the PCR amplification of cytokine mRNAs isolated from mucosal leishmaniasis (Panel A) and cutaneous leishmaniasis (panel B) patient PBMC before and after stimulation with representative polypeptides of the present invention. Lanes O and - indicate the level of PCR products at the initiation of culture and after 72 hours of culture, respectively, in the absence of added polypeptide; lanes Lb, 83a and 83b indicate the level of PCR products following culturing of PBMC with *L. braziliensis* lysate, and the *Leishmania* antigens Lbhsp83a and Lbhsp83b, respectively.

Figure 10 presents a comparison of the levels of interferon- γ (panel A) and TNF- α (panel B) in the supernatants of 72 hour PBMC cultures from *Leishmania*-

infected and control individuals in response to stimulation with parasite lysate or the indicated polypeptides.

Figure 11 illustrates the levels of IL-10 p40 (in pg/mL) in the supernatant of PBMC cultures from *L. braziliensis*-infected individuals and uninfected controls 72 hours following stimulation with parasite promastigote lysate (Lb), Lbhsp83a or Lbhsp83b.

Figure 12 presents the reactivities of sera from *L. braziliensis* infected-patients with representative polypeptides of the present invention in a standard ELISA. Values are expressed as absorbance at 405 nm.

Figures 13A and 13B illustrate the level of secreted IL-4 and IFN- γ (in pg/mL) stimulated in mouse lymph node cultures by the addition of representative polypeptides of the present invention.

Figure 14 shows the level of IFN- γ (in pg/mL) secreted by *Leishmania*-infected and uninfected human PBMC stimulated by the *Leishmania* antigen M15, as compared to the levels stimulated by *L. major* lysate and L-Rack, an antigen that does not appear to be recognized by *Leishmania*-infected humans.

Figure 15 shows the level of IFN- γ (in pg/mL) secreted by infected and uninfected human PBMC stimulated by soluble *Leishmania* antigens (S antigens), as compared to the levels stimulated by *L. major* lysate and L-Rack.

Figure 16 illustrates the proliferation of murine lymph node cultures stimulated by the addition of representative polypeptides of the present invention. Values are expressed as cpm.

Figure 17 shows the proliferation of human PBMC, prepared from *Leishmania*-immune and uninfected individuals, stimulated by M15 as compared to the proliferation stimulated by *L. major* lysate and L-Rack. Values are expressed as cpm.

Figure 18 illustrates the proliferation of human PBMC, prepared from *Leishmania*-infected and uninfected individuals, stimulated by soluble *Leishmania* antigens as compared to the proliferation stimulated by culture medium, *L. major* lysate and L-Rack. Values are expressed as cpm.

Figure 19 presents a comparison of a Lbhsp83 sequence (SEQ ID NO:6) with homologous sequences from *L. amazonensis* (Lahsp83) (SEQ ID NO:16), *T. cruzi* (Tchsp83) (SEQ ID NO:17) and humans (Huhsp89) (SEQ ID NO:18).

Figure 20 illustrates the reactivity of rabbit sera raised against soluble *Leishmania* antigens with *Leishmania* promastigote lysate (lane 1) and soluble *Leishmania* antigens (lane 2).

Figure 21 shows the cDNA and predicted amino acid sequence for the *Leishmania* antigen LmsplA.

Figure 22 shows a Southern blot of genomic DNA from *L. major* digested with a panel of restriction enzymes (lanes 1 to 7) and six other *Leishmania* species digested with PstI (lanes 8 to 13) probed with the full-length cDNA insert of LmsplA.

Figure 23 shows a Southern blot of genomic DNA from *L. major* digested with a panel of restriction enzymes, six other *Leishmania* species digested with PstI and the infectious pathogens *T. cruzi* and *T. brucei*, probed with the full-length cDNA insert of the *Leishmania* antigen MAPS-1A.

Figure 24 illustrates the proliferation of PBMC isolated from uninfected individuals, patients with active mucosal leishmaniasis and patients post kala-azar infection, stimulated by MAPS-1A.

Figure 25 illustrates the proliferation of murine lymph node cultures stimulated by MAPS-1A.

Figure 26 illustrates the reactivity of MAPS-1A with sera from human leishmaniasis patients.

Figure 27 illustrates the reactivity of MAPS-1A with sera from mice immunized against and/or infected with leishmaniasis.

Figure 28 illustrates the effectiveness of immunization with either soluble *Leishmania* antigens or a mixture of Ldp23, LbeiF4A and M15 plus adjuvant in conferring protection against infection (as measured by footpad swelling) in a murine leishmaniasis model system, as compared to the administration of adjuvant alone.

Figure 29 illustrates the effectiveness of immunization with MAPS-1A plus adjuvant in conferring protection against infection (as measured by footpad swelling) in a murine leishmaniasis model system, as compared to the administration of adjuvant alone.

5 Figures 30A and B illustrate the proliferation of murine lymph node cultures stimulated with either LcgSP8, LcgSP10 or LcgSP3.

DETAILED DESCRIPTION OF THE INVENTION

As noted above, the present invention is generally directed to
10 compositions and methods for preventing, treating and detecting leishmaniasis, as well as for stimulating immune responses in patients. The compositions of the subject invention include polypeptides that comprise at least an immunogenic portion of a *Leishmania* antigen, or a variant of such an antigen that differs only in conservative substitutions and/or modifications. In one preferred embodiment, compositions of the
15 present invention include multiple polypeptides selected so as to provide enhanced protection against a variety of *Leishmania* species.

Polypeptides within the scope of the present invention include, but are not limited to, polypeptides comprising immunogenic portions of *Leishmania* antigens comprising the sequences recited in SEQ ID NO:2 (referred to herein as M15), SEQ ID
20 NO:4 (referred to herein as Ldp23), SEQ ID NO:6 (referred to herein as Lbhsp83), SEQ ID NO:8 (referred to herein as Lt-210), SEQ ID NO:10 (referred to herein as LbeIF4A), SEQ ID NO: 20 (referred to herein as Lmsp1a), SEQ ID NO: 22 (referred to herein as Lmsp9a), SEQ ID NOs: 24 and 26 (referred to herein as MAPS-1A), and SEQ ID NO: 36-42, 49-53 and 55. As used herein, the term "polypeptide" encompasses amino acid
25 chains of any length, including full length proteins (*i.e.*, antigens), wherein the amino acid residues are linked by covalent bonds. Thus, a polypeptide comprising an immunogenic portion of one of the above antigens may consist entirely of the immunogenic portion, or may contain additional sequences. The additional sequences may be derived from the native *Leishmania* antigen or may be heterologous, and such
30 sequences may (but need not) be immunogenic. An antigen "having" a particular

sequence is an antigen that contains, within its full length sequence, the recited sequence. The native antigen may, or may not, contain additional amino acid sequence.

An immunogenic portion of a *Leishmania* antigen is a portion that is capable of eliciting an immune response (*i.e.*, cellular and/or humoral) in a presently or previously *Leishmania*-infected patient (such as a human or a dog) and/or in cultures of lymph node cells or peripheral blood mononuclear cells (PBMC) isolated from presently or previously *Leishmania*-infected individuals. The cells in which a response is elicited may comprise a mixture of cell types or may contain isolated component cells (including, but not limited to, T-cells, NK cells, macrophages, monocytes and/or B cells). In particular, immunogenic portions are capable of inducing T-cell proliferation and/or a dominantly Th1-type cytokine response (*e.g.*, IL-2, IFN- γ , and/or TNF- α production by T-cells and/or NK cells; and/or IL-12 production by monocytes, macrophages and/or B cells). Immunogenic portions of the antigens described herein may generally be identified using techniques known to those of ordinary skill in the art, including the representative methods provided herein.

The compositions and methods of the present invention also encompass variants of the above polypeptides. A polypeptide "variant," as used herein, is a polypeptide that differs from the native antigen only in conservative substitutions and/or modifications, such that the ability of the polypeptide to include an immune response is retained. Polypeptide variants preferably exhibit at least about 70%, more preferably at least about 90% and most preferably at least about 95% identity to the identified polypeptides. Alternatively, such variants may be identified by modifying one of the above polypeptide sequences and evaluating the immunogenic properties of the modified polypeptide using, for example, the representative procedures described herein.

A "conservative substitution" is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydropathic nature of the polypeptide to be substantially unchanged. In general, the following groups of amino acids represent conservative changes: (1) ala, pro, gly, glu, asp, gln,

asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his.

Variants may also (or alternatively) be modified by, for example, the deletion or addition of amino acids that have minimal influence on the immunogenic properties, secondary structure and hydrophobic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification or identification of the polypeptide (e.g., poly-His), or to enhance binding of the polypeptide to a solid support. For example, a polypeptide may be conjugated to an immunoglobulin Fc region.

A nucleotide "variant" is a sequence that differs from the recited nucleotide sequence in having one or more nucleotide deletions, substitutions or additions. Such modifications may be readily introduced using standard mutagenesis techniques, such as oligonucleotide-directed site-specific mutagenesis as taught, for example, by Adelman et al. (*DNA*, 2:183, 1983). Nucleotide variants may be naturally occurring allelic variants, or non-naturally occurring variants. Variant nucleotide sequences preferably exhibit at least about 70%, more preferably at least about 80% and most preferably at least about 90% identity to the recited sequence. Such variant nucleotide sequences will generally hybridize to the recited nucleotide sequence under stringent conditions. As used herein, "stringent conditions" refers to prewashing in a solution of 6X SSC, 0.2% SDS; hybridizing at 65 °C, 6X SSC, 0.2% SDS overnight; followed by two washes of 30 minutes each in 1X SSC, 0.1% SDS at 65 °C and two washes of 30 minutes each in 0.2X SSC, 0.1% SDS at 65 °C.

"Polypeptides" as described herein also include combination polypeptides. A "combination polypeptide" is a polypeptide comprising at least one of the above immunogenic portions and one or more additional immunogenic *Leishmania* sequences, which are joined via a peptide linkage into a single amino acid chain. The sequences may be joined directly (i.e., with no intervening amino acids) or may be

joined by way of a linker sequence (e.g., Gly-Cys-Gly) that does not significantly diminish the immunogenic properties of the component polypeptides.

In general, *Leishmania* antigens having immunogenic properties, and DNA sequences encoding such antigens, may be prepared using any of a variety of procedures from one or more *Leishmania* species including, but not limited to, *L. donovani*, *L. chagasi*, *L. infantum*, *L. major*, *L. amazonensis*, *L. braziliensis*, *L. panamensis*, *L. mexicana*, *L. tropica*, and *L. guyanensis*. Such species are available, for example, from the American Type Culture Collection (ATCC), Rockville, MD. For example, peptides isolated from MHC class II molecules of macrophages infected with a *Leishmania* species may be used to rescue the corresponding *Leishmania* donor antigens. MHC class II molecules are expressed mainly by cells of the immune system, including macrophages. These molecules present peptides, which are usually 13-17 amino acids long, derived from foreign antigens that are degraded in cellular vesicles. The bound peptide antigens are then recognized by CD4 T-cells. Accordingly, foreign peptides isolated from MHC class II molecules of, for example, *Leishmania*-infected murine macrophages may be used to identify immunogenic *Leishmania* proteins.

Briefly, peptides derived from *Leishmania* antigens may be isolated by comparing the reverse phase HPLC profile of peptides extracted from infected macrophages with the profile of peptides extracted from uninfected cells. Peptides giving rise to distinct HPLC peaks unique to infected macrophages may then be sequenced using, for example, Edman chemistry as described in Edman and Berg, *Eur J. Biochem*, 80:116-132 (1967). A DNA fragment corresponding to a portion of a *Leishmania* gene encoding the peptide may then be amplified from a *Leishmania* cDNA library using an oligonucleotide sense primer derived from the peptide sequence and an oligo dT antisense primer. The resulting DNA fragment may then be used as a probe to screen a *Leishmania* library for a full length cDNA or genomic clone that encodes the *Leishmania* antigen. Such screens may generally be performed using techniques well known to those of ordinary skill in the art, such as those described in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY (1989).

This approach may be used to identify a 23 kD *Leishmania donovani* antigen (referred to herein as Ldp23). The sequence of a DNA molecule encoding Ldp23 is provided in SEQ ID NO:3 and the amino acid sequence of Ldp23 is provided in SEQ ID NO:4. Using the methods described herein, Ldp23 has been shown to induce a Th1 immune response in T-cells prepared from *Leishmania*-infected mice.

Alternatively, a *Leishmania* cDNA or genomic expression library may be screened with serum from a *Leishmania*-infected individual, using techniques well known to those of ordinary skill in the art. DNA molecules encoding reactive antigens may then be used to express the recombinant antigen for purification. The immunogenic properties of the purified *Leishmania* antigens may then be evaluated using, for example the representative methods described herein.

For example, sera from *Leishmania*-infected mice may be used to screen a cDNA library prepared from *Leishmania* amastigotes. Reactive clones may then be expressed and recombinant proteins assayed for the ability to stimulate T-cells or NK cells derived from *Leishmania*-immune individuals (*i.e.*, individuals having evidence of infection, as documented by positive serological reactivity with *Leishmania*-specific antibodies and/or a *Leishmania*-specific DTH response, without clinical symptoms of leishmaniasis). This procedure may be used to obtain a recombinant DNA molecule encoding the *Leishmania* antigen designated M15. The sequence of such a DNA molecule is provided in SEQ ID NO:1, and the amino acid sequence of the encoded protein is provided in SEQ ID NO:2.

A similar approach may be used to isolate a genomic DNA molecule encoding an immunogenic *Leishmania braziliensis* antigen, referred to herein as Lbhsp83. More specifically, a genomic clone encoding Lbhsp83 may be isolated by screening a *L. braziliensis* expression library with sera from a *Leishmania*-infected individual. The DNA encoding Lbhsp83 is homologous to the gene encoding the eukaryotic 83 kD heat shock protein. The sequence of a DNA molecule encoding nearly all of Lbhsp83 is presented in SEQ ID NO:5, and the encoded amino acid sequence is provided in SEQ ID NO:6. Using the methods described below, Lbhsp83 has been found to stimulate proliferation, and a mixed Th1 and Th2 cytokine profile, in

PBMC isolated from *L. braziliensis*-infected patients. Accordingly, Lbhsp83 is an immunogenic *Leishmania* antigen. Regions of Lbhsp83 that are not conserved with the mammalian gene have been found to be particularly potent for T-cell stimulation and antibody binding. Such regions may be identified, for example, by visual inspection of the sequence comparison provided in Figure 19.

This approach may also be used to isolate a DNA molecule encoding a 210 kD immunogenic *L. tropica* antigen, referred to herein as Lt-210. The preparation and characterization of Lt-210, and immunogenic portions thereof (such as Lt-1 and immunogenic repeat and non-repeat sequences), is described in detail in U.S. Patent Application Serial No. 08/511,872, filed August 4, 1995. The sequence of a DNA molecule encoding Lt-1 is provided in SEQ ID NO:7 and the encoded amino acid sequence is presented in SEQ ID NO:8.

The above approach may further be used to isolate a DNA molecule encoding a *L. braziliensis* antigen referred to herein as LbeIF4A. Briefly, such a clone may be isolated by screening a *L. braziliensis* expression library with sera obtained from a patient afflicted with mucosal leishmaniasis, and analyzing the reactive antigens for the ability to stimulate proliferative responses and preferential Th1 cytokine production in PBMC isolated from *Leishmania*-infected patients, as described below. The preparation and characterization of LbeIF4A is described in detail in U.S. Patent Application Serial Nos. 08/454,036 and 08/488,386, which are continuations-in-part of U.S. Patent Application Serial No. 08/232,534, filed April 22, 1994. The sequence of a DNA molecule encoding LbeIF4A is provided in SEQ ID NO:9 and the encoded amino acid sequence is presented in SEQ ID NO:10. Homologs of LbeIF4A, such as that found in *L. major*, may also be isolated using this approach, and are within the scope of the present invention.

Compositions of the present invention may also, or alternatively, contain soluble *Leishmania* antigens. As used herein, "soluble *Leishmania* antigens" refers to a mixture of at least 8 different *Leishmania* antigens that may be isolated from the supernatant of *Leishmania* promastigotes of any species grown for 8-12 hours in protein-free medium. Briefly, the organisms are grown to late log phase in complex

medium with serum until they reach a density of $2-3 \times 10^7$ viable organisms per mL of medium. The organisms are thoroughly washed to remove medium components and resuspended at $2-3 \times 10^7$ viable organisms per mL of defined serum-free medium consisting of equal parts RPMI 1640 and medium 199, both from Gibco BRL, Gaithersburg, MD. After 8-12 hours, the supernatant containing soluble *Leishmania* antigens is removed, concentrated 10 fold and dialyzed against phosphate-buffered saline for 24 hours. The presence of at least eight different antigens within the mixture of *Leishmania* antigens may be confirmed using SDS-PAGE (i.e., through the observation of at least 8 different bands). The immunogenic properties of the soluble *Leishmania* antigens may be confirmed by evaluating the ability of the preparation to elicit an immune response in cultures of lymph node cells and/or peripheral blood mononuclear cells (PBMC) isolated from presently or previously *Leishmania*-infected individuals. Such an evaluation may be performed as described below.

Individual antigens present within the mixture of soluble *Leishmania* antigens may be isolated by immunizing mice or rabbits with *Leishmania* culture supernatant, containing soluble antigens, and employing the resultant sera to screen a *Leishmania* cDNA expression library as described in detail below. This procedure may be used to isolate recombinant DNA molecules encoding the *L. major* antigens referred to herein as Lmsp1a, Lmsp9a and MAPS-1A. DNA sequences encoding Lmsp1a, Lmsp9a and MAPS-1A are provided in SEQ ID NO: 19, 21 and 23, respectively, with the corresponding predicted amino acid sequences being presented in SEQ ID NO: 20, 22 and 24, respectively. Similarly, sera from mice or rabbits immunized with *L. major* culture supernatant may be used to screen an *L. major* genomic DNA library. As detailed below, this procedure may be used to isolate DNA molecules encoding the *L. major* antigens referred to herein as LmgSP1, LmgSP3, LmgSP5, LmgSP8, LmgSP9, LmgSP13, LmgSP19, and DNA molecules encoding the *L. chagasi* antigens LcgSP1, LcgSP3, LcgSP4, LcgSP8, and LcgSP10. The DNA sequences encoding these antigens are provided in SEQ ID NO:29-35 and 44-48, respectively, with the corresponding amino acid sequences being provided in SEQ ID NO: 36-42 and 49-53. The *L. major* antigens referred to herein as 1G6-34, 1E6-44, 4A5-63, 1B11-39, 2A10-37, 4G2-83,

4H6-41 and 8G3-100 may be isolated by means of CD4+ T cell expression cloning as described below. DNA sequences encoding these antigens are provided in SEQ ID NO: 72-79, respectively, with the corresponding predicted amino acid sequences being provided in SEQ ID NO: 80-87. The immunogenic properties of the isolated *Leishmania* antigens may be evaluated using, for example, the representative methods described herein.

Regardless of the method of preparation, the antigens described herein are immunogenic. In other words, the antigens (and immunogenic portions thereof) are capable of eliciting an immune response in cultures of lymph node cells and/or peripheral blood mononuclear cells (PBMC) isolated from presently or previously *Leishmania*-infected individuals. More specifically, the antigens, and immunogenic portions thereof, have the ability to induce T-cell proliferation and/or to elicit a dominantly Th1-type cytokine response (e.g., IL-2, IFN- γ , and/or TNF- α production by T-cells and/or NK cells; and/or IL-12 production by monocytes, macrophages and/or B cells) in cells isolated from presently or previously *Leishmania*-infected individuals. A *Leishmania*-infected individual may be afflicted with a form of leishmaniasis (such as subclinical, cutaneous, mucosal or active visceral) or may be asymptomatic. Such individuals may be identified using methods known to those of ordinary skill in the art. Individuals with leishmaniasis may be identified based on clinical findings associated with at least one of the following: isolation of parasite from lesions, a positive skin test with *Leishmania* lysate or a positive serological test. Asymptomatic individuals are infected individuals who have no signs or symptoms of the disease. Such individuals can be identified based on a positive serological test and/or skin test with *Leishmania* lysate.

The term "PBMC," which refers to a preparation of nucleated cells consisting primarily of lymphocytes and monocytes that are present in peripheral blood, encompasses both mixtures of cells and preparations of one or more purified cell types. PBMC may be isolated by methods known to those in the art. For example, PBMC may be isolated by density centrifugation through, for example, Ficoll™ (Winthrop Laboratories, New York). Lymph node cultures may generally be prepared by

immunizing BALB/c mice (*e.g.*, in the rear foot pad) with *Leishmania* promastigotes emulsified in complete Freund's adjuvant. The draining lymph nodes may be excised following immunization and T-cells may be purified in an anti-mouse Ig column to remove the B cells, followed by a passage through a Sephadex G10 column to remove the macrophages. Similarly, lymph node cells may be isolated from a human following
5 biopsy or surgical removal of a lymph node.

The ability of a polypeptide (*e.g.*, a *Leishmania* antigen or a portion or other variant thereof) to induce a response in PBMC or lymph node cell cultures may be evaluated by contacting the cells with the polypeptide and measuring a suitable
10 response. In general, the amount of polypeptide that is sufficient for the evaluation of about 2×10^5 cells ranges from about 10 ng to about 100 μ g, and preferably is about 1-10 μ g. The incubation of polypeptide with cells is typically performed at 37°C for about 1-3 days. Following incubation with polypeptide, the cells are assayed for an appropriate response. If the response is a proliferative response, any of a variety of
15 techniques well known to those of ordinary skill in the art may be employed. For example, the cells may be exposed to a pulse of radioactive thymidine and the incorporation of label into cellular DNA measured. In general, a polypeptide that results in at least a three fold increase in proliferation above background (*i.e.*, the proliferation observed for cells cultured without polypeptide) is considered to be able to
20 induce proliferation.

Alternatively, the response to be measured may be the secretion of one or more cytokines (such as interferon- γ (IFN- γ), interleukin-4 (IL-4), interleukin-12 (p70 and/or p40), interleukin-2 (IL-2) and/or tumor necrosis factor- α (TNF- α)) or the change in the level of mRNA encoding one or more specific cytokines. In particular,
25 the secretion of interferon- γ , interleukin-2, tumor necrosis factor- α and/or interleukin-12 is indicative of a Th1 response, which is responsible for the protective effect against *Leishmania*. Assays for any of the above cytokines may generally be performed using methods known to those of ordinary skill in the art, such as an enzyme-linked immunosorbent assay (ELISA). Suitable antibodies for use in such assays may be
30 obtained from a variety of sources such as Chemicon, Temucula, CA and PharMingen,

San Diego, CA, and may generally be used according to the manufacturer's instructions. The level of mRNA encoding one or more specific cytokines may be evaluated by, for example, amplification by polymerase chain reaction (PCR). In general, a polypeptide that is able to induce, in a preparation of about $1-3 \times 10^5$ cells, the production of 30 pg/mL of IL-12, IL-4, IFN- γ , TNF- α or IL-12 p40, or 10 pg/mL of IL-12 p70, is considered able to stimulate production of a cytokine.

Immunogenic portions of the antigens described herein may be prepared and identified using well known techniques, such as those summarized in Paul, Fundamental Immunology, 3rd ed., 243-247 (Raven Press, 1993) and references cited therein. Such techniques include screening polypeptides derived from the native antigen for immunogenic properties using, for example, the representative techniques described herein. An immunogenic portion of a polypeptide is a portion that, within such representative assays, generates an immune response (e.g., proliferation and/or cytokine production) that is substantially similar to that generated by the full length antigen. In other words, an immunogenic portion of an antigen may generate at least about 25%, and preferably at least about 50%, of the response generated by the full length antigen in the model assays described herein.

Portions and other variants of immunogenic *Leishmania* antigens may be generated by synthetic or recombinant means. Synthetic polypeptides having fewer than about 100 amino acids, and generally fewer than about 50 amino acids, may be generated using techniques well known to those of ordinary skill in the art. For example, such polypeptides may be synthesized using any of the commercially available solid-phase techniques, such as the Merrifield solid-phase synthesis method, where amino acids are sequentially added to a growing amino acid chain. See Merrifield, *J. Am. Chem. Soc.* 85:2149-2146, 1963. Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Perkin Elmer/Applied BioSystems Division, Foster City, CA, and may be operated according to the manufacturer's instructions.

Recombinant polypeptides containing portions and/or variants of a native antigen may be readily prepared from a DNA sequence encoding the antigen.

For example, supernatants from suitable host/vector systems which secrete recombinant protein into culture media may be first concentrated using a commercially available filter. Following concentration, the concentrate may be applied to a suitable purification matrix such as an affinity matrix or an ion exchange resin. Finally, one or more reverse phase HPLC steps can be employed to further purify a recombinant protein.

In general, any of a variety of expression vectors known to those of ordinary skill in the art may be employed to express recombinant polypeptides of this invention. Expression may be achieved in any appropriate host cell that has been transformed or transfected with an expression vector containing a DNA molecule that encodes a recombinant polypeptide. Suitable host cells include prokaryotes, yeast and higher eukaryotic cells. Preferably, the host cells employed are *E. coli*, yeast or a mammalian cell line such as COS or CHO. The DNA sequences expressed in this manner may encode naturally occurring antigens, portions of naturally occurring antigens, or other variants thereof. For example, variants of a native antigen may generally be prepared using standard mutagenesis techniques, such as oligonucleotide-directed site-specific mutagenesis, and sections of the DNA sequence may be removed to permit preparation of truncated polypeptides.

In another aspect, the present invention provides epitope repeat sequences, or antigenic epitopes, of a *Leishmania* antigen, together with polypeptides comprising at least two such contiguous antigenic epitopes. As used herein an "epitope" is a portion of an antigen that reacts with sera from *Leishmania*-infected individuals (i.e. an epitope is specifically bound by one or more antibodies present in such sera). As discussed above, epitopes of the antigens described in the present application may be generally identified using techniques well known to those of skill in the art.

In one embodiment, antigenic epitopes of the present invention comprise an amino acid sequence provided in SEQ ID NO:43, 56, 57 or 58. As discussed in more detail below, antigenic epitopes provided herein may be employed in the diagnosis and treatment of *Leishmania* infection, either alone or in combination with other

Leishmania antigens or antigenic epitopes. Antigenic epitopes and polypeptides comprising such epitopes may be prepared by synthetic means, as described generally above and in detail in Example 15.

In certain aspects of the present invention, described in detail below, the polypeptides, antigenic epitopes and/or soluble *Leishmania* antigens may be incorporated into pharmaceutical compositions or vaccines. For clarity, the term "polypeptide" will be used when describing specific embodiments of the inventive therapeutic compositions and diagnostic methods. However, it will be clear to one of skill in the art that the antigenic epitopes of the present invention may also be employed in such compositions and methods.

Pharmaceutical compositions comprise one or more polypeptides, each of which may contain one or more of the above sequences (or variants thereof), and a physiologically acceptable carrier. Vaccines comprise one or more of the above polypeptides and a non-specific immune response enhancer, such as an adjuvant (e.g., LbeIF4A, interleukin-12 or other cytokines) or a liposome (into which the polypeptide is incorporated). Vaccines may additionally contain a delivery vehicle, such as a biodegradable microsphere (disclosed, for example, in U.S. Patent Nos. 4,897,268 and 5,075,109). Pharmaceutical compositions and vaccines within the scope of the present invention may also contain other *Leishmania* antigens, either incorporated into a combination polypeptide or present within one or more separate polypeptides.

Alternatively, a pharmaceutical composition or vaccine may contain DNA encoding one or more of the polypeptides as described above, such that the polypeptide is generated *in situ*. In such pharmaceutical compositions and vaccines, the DNA may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid expression systems, bacteria and viral expression systems. Appropriate nucleic acid expression systems contain the necessary DNA sequences for expression in the patient (such as a suitable promoter and terminating signal). Bacterial delivery systems involve the administration of a bacterium (such as *Bacillus-Calmette-Guerrin*) that expresses an immunogenic portion of the polypeptide on its cell surface. In a preferred embodiment, the DNA may be

introduced using a viral expression system (e.g., vaccinia or other pox virus, retrovirus, or adenovirus), which may involve the use of a non-pathogenic (defective), replication competent virus. Techniques for incorporating DNA into such expression systems are well known to those of ordinary skill in the art. The DNA may also be "naked," as
5 described, for example, in Ulmer et al., *Science* 259:1745-1749 (1993) and reviewed by Cohen, *Science* 259:1691-1692 (1993). The uptake of naked DNA may be increased by coating the DNA onto biodegradable beads, which are efficiently transported into the cells.

While any suitable carrier known to those of ordinary skill in the art may
10 be employed in the pharmaceutical compositions of this invention, the type of carrier will vary depending on the mode of administration. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a fat, a wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum,
15 cellulose, glucose, sucrose, and magnesium carbonate, may be employed. Biodegradable microspheres (e.g., polylactic galactide) may also be employed as carriers for the pharmaceutical compositions of this invention. Suitable biodegradable microspheres are disclosed, for example, in U.S. Patent Nos. 4,897,268 and 5,075,109.

Any of a variety of adjuvants may be employed in the vaccines of this
20 invention to nonspecifically enhance the immune response. Most adjuvants contain a substance designed to protect the antigen from rapid catabolism, such as aluminum hydroxide or mineral oil, and a nonspecific stimulator of immune responses, such as lipid A, *Bordella pertussis* or *Mycobacterium tuberculosis*. Suitable adjuvants are commercially available as, for example, Freund's Incomplete Adjuvant and Complete
25 Adjuvant (Difco Laboratories, Detroit, MI), Merck Adjuvant 65 (Merck and Company, Inc., Rahway, NJ), alum, biodegradable microspheres, monophosphoryl lipid A and quil A. Preferred adjuvants include LbeIF4A, IL-12 and other cytokines such as IFN- γ or granulocyte-macrophage colony stimulating factor (GM-CSF). By virtue of its ability to induce an exclusive Th1 immune response, the use of LbeIF4A, and variants thereof,
30 as an adjuvant in the vaccines of the present invention is particularly preferred.

In one preferred embodiment, compositions of the present invention include multiple polypeptides selected so as to provide enhanced protection against a variety of *Leishmania* species. Such polypeptides may be selected based on the species of origin of the native antigen or based on a high degree of conservation of amino acid sequence among different species of *Leishmania*. A combination of individual polypeptides may be particularly effective as a prophylactic and/or therapeutic vaccine because (1) stimulation of proliferation and/or cytokine production by individual polypeptides may be additive, (2) stimulation of proliferation and/or cytokine production by individual polypeptides may be synergistic, (3) individual polypeptides may stimulate cytokine profiles in such a way as to be complementary to each other and/or (4) individual polypeptides may be complementary to one another when certain of them are expressed more abundantly on the individual species or strain of *Leishmania* responsible for infection. A preferred combination contains polypeptides that comprise immunogenic portions of M15, Ldp23, Lbhsp83, Lt-1 and LbeIF4A. Alternatively, or in addition, the combination may include one or more polypeptides comprising immunogenic portions of other *Leishmania* antigens disclosed herein, and/or soluble *Leishmania* antigens.

The above pharmaceutical compositions and vaccines may be used, for example, to induce protective immunity against *Leishmania* in a patient, such as a human or a dog, to prevent leishmaniasis. Appropriate doses and methods of administration for this purposes are described in detail below.

The pharmaceutical compositions and vaccines described herein may also be used to stimulate an immune response, which may be cellular and/or humoral, in a patient. For *Leishmania*-infected patients, the immune responses that may be generated include a preferential Th1 immune response (*i.e.*, a response characterized by the production of the cytokines interleukin-1, interleukin-2, interleukin-12 and/or interferon- γ , as well as tumor necrosis factor- α). For uninfected patients, the immune response may be the production of interleukin-12 and/or interleukin-2, or the stimulation of gamma delta T-cells. In either category of patient, the response stimulated may include IL-12 production. Such responses may also be elicited in

biological samples of PBMC or components thereof derived from *Leishmania*-infected or uninfected individuals. As noted above, assays for any of the above cytokines may generally be performed using methods known to those of ordinary skill in the art, such as an enzyme-linked immunosorbent assay (ELISA).

5 Suitable pharmaceutical compositions and vaccines for use in this aspect of the present invention are those that contain at least one polypeptide comprising an immunogenic portion of a *Leishmania* antigen disclosed herein (or a variant thereof). Preferably, the polypeptides employed in the pharmaceutical compositions and vaccines are complementary, as described above. Soluble *Leishmania* antigens, with or without
10 additional polypeptides, may also be employed.

 The pharmaceutical compositions and vaccines described herein may also be used to treat a patient afflicted with a disease responsive to IL-12 stimulation. The patient may be any warm-blooded animal, such as a human or a dog. Such diseases include infections (which may be, for example, bacterial, viral or protozoan) or diseases
15 such as cancer. In one embodiment, the disease is leishmaniasis, and the patient may display clinical symptoms or may be asymptomatic. In general, the responsiveness of a particular disease to IL-12 stimulation may be determined by evaluating the effect of treatment with a pharmaceutical composition or vaccine of the present invention on clinical correlates of immunity. For example, if treatment results in a heightened Th1
20 response or the conversion of a Th2 to a Th1 profile, with accompanying clinical improvement in the treated patient, the disease is responsive to IL-12 stimulation. Polypeptide administration may be as described below, or may extend for a longer period of time, depending on the indication. Preferably, the polypeptides employed in the pharmaceutical compositions and vaccines are complementary, as described above.
25 A particularly preferred combination contains polypeptides that comprise immunogenic portions of M15, Ldp23, Lbhsp83, Lt-1 and LbeIF4A, Lmsp1a, Lmsp9a, and MAPS-1A. Soluble *Leishmania* antigens, with or without additional polypeptides, may also be employed.

 Routes and frequency of administration, as well as dosage, for the above
30 aspects of the present invention will vary from individual to individual and may parallel

those currently being used in immunization against other infections, including protozoan, viral and bacterial infections. In general, the pharmaceutical compositions and vaccines may be administered by injection (*e.g.*, intracutaneous, intramuscular, intravenous or subcutaneous), intranasally (*e.g.*, by aspiration) or orally. Between 1 and 5 12 doses may be administered over a 1 year period. For therapeutic vaccination (*i.e.*, treatment of an infected individual), 12 doses are preferably administered, at one month intervals. For prophylactic use, 3 doses are preferably administered, at 3 month intervals. In either case, booster vaccinations may be given periodically thereafter. Alternate protocols may be appropriate for individual patients. A suitable dose is an 10 amount of polypeptide or DNA that, when administered as described above, is capable of raising an immune response in an immunized patient sufficient to protect the patient from leishmaniasis for at least 1-2 years. In general, the amount of polypeptide present in a dose (or produced *in situ* by the DNA in a dose) ranges from about 100 ng to about 1mg per kg of host, typically from about 10 μ g to about 100 μ g. Suitable dose sizes 15 will vary with the size of the patient, but will typically range from about 0.1 mL to about 5 mL.

In another aspect, this invention provides methods for using one or more of the polypeptides described above to diagnose *Leishmania* infection in a patient using a skin test. As used herein, a "skin test" is any assay performed directly on a patient in 20 which a delayed-type hypersensitivity (DTH) reaction (such as induration and accompanying redness) is measured following intradermal injection of one or more polypeptides as described above. Such injection may be achieved using any suitable device sufficient to contact the polypeptide or polypeptides with dermal cells of the patient, such as a tuberculin syringe or 1 mL syringe. Preferably, the reaction is 25 measured at least 48 hours after injection, more preferably 72 hours after injection.

The DTH reaction is a cell-mediated immune response, which is greater in patients that have been exposed previously to a test antigen (*i.e.*, an immunogenic portion of a polypeptide employed, or a variant thereof). The response may be measured visually, using a ruler. In general, induration that is greater than about 0.5 cm in 30 diameter, preferably greater than about 1.0 cm in diameter, is a positive response,

indicative of *Leishmania* infection, which may or may not be manifested as an active disease.

The polypeptides of this invention are preferably formulated, for use in a skin test, as pharmaceutical compositions containing at least one polypeptide and a physiologically acceptable carrier, as described above. Such compositions typically contain one or more of the above polypeptides in an amount ranging from about 1 μ g to 100 μ g, preferably from about 10 μ g to 50 μ g in a volume of 0.1 mL. Preferably, the carrier employed in such pharmaceutical compositions is a saline solution with appropriate preservatives, such as phenol and/or Tween 80™.

The inventive polypeptides may also be employed in combination with one or more known *Leishmania* antigens in the diagnosis of leishmaniasis, using, for example, the skin test described above. Preferably, individual polypeptides are chosen in such a way as to be complementary to each other. Examples of known *Leishmania* antigens which may be usefully employed in conjunction with the inventive polypeptides include K39 (Burns et al., Proc. Natl. Acad. Sci. USA, 1993 90:775-779).

The following Examples are offered by way of illustration and not by way of limitation.

EXAMPLES

EXAMPLE 1

PREPARATION OF M15

This Example illustrates the preparation of a *Leishmania* antigen M15, having the sequence provided in SEQ ID NO:2.

An *L. major* (Friedlan strain) amastigote cDNA expression library prepared in the λ ZAP II vector (Stratagene, La Jolla, CA) was screened according to manufacturer's instructions using sera obtained from *L. major* infected BALB/c mice (8 weeks post inoculation). Approximately 40,000 plaques were screened and four clones expressing reactive antigens were purified to homogeneity by two subsequent rounds of low density screening. Bluescript phagemid inserts were excised from positive clones

for further analysis. An *EcoRI/SstII* restriction fragment from the 5' end of one partial cDNA insert isolated during first round screening (pLmal-1) was subsequently used as a probe to rescreen for clones containing full length cDNA inserts. The probe was labeled to high specific activity (10^9 cpm/ μ g) with [32 P]dCTP using the random primer method and was used to screen 10,000 plaques of the *L. major* expression library described above. Positive clones were compared by restriction enzyme digestion and the clone with the largest insert (pf11-1) was chosen for subsequent analysis.

DNA sequence analyses were performed on an Applied Biosystems automated sequencer using Taq polymerase and dye coupled ddNTP terminators or dye-labeled sequencing primers. The complete sequence of the 2685 bp insert was determined using a combination of primer-directed sequencing and by sequencing a series of overlapping Exonuclease III deletion subclones generated using the Erase-a-base system (Promega, Madison, WI). The sequence of this insert is provided in SEQ ID NO:1, and the deduced amino acid sequence is provided in SEQ ID NO:2.

The complete insert of clone pf11-1 was excised by digestion with *BamHI/KpnI* and was subcloned in frame into *BamHI/KpnI* digested pQE31 (QUIAGEN) to generate the construct pM151A. *E. coli* containing this construct inducibly expressed high levels of the *L. major* antigen encoded by pf11-1 (designated as M15) with the addition of a 6-histidine tag at the amino terminus. Large volume cultures (500 ml) of *E. coli* host cells containing the pM151A construct were induced to express recombinant protein by the addition of 2mM IPTG at mid-log phase of growth. Growth was continued for 4 to 5 hours and bacteria were then pelleted and washed once with cold PBS. Bacteria were resuspended in 20 ml of lysis buffer (50 mM Na_2HPO_4 , pH 8.0, 300 mM NaCl, 10 mM β -mercaptoethanol) containing 20 mg of lysozyme and were lysed by a 1 hour incubation at 4°C followed by brief sonication. Insoluble material was removed by centrifugation at 10,000xg for 10 minutes and although the recombinant protein was found to be evenly distributed between the soluble and insoluble fractions the insoluble material was discarded at this point. Recombinant protein containing the amino terminal histidine tag was affinity purified using Ni-NTA resin (QUIAGEN) according to the manufacturer's recommendations. Briefly, 8 ml of

Ni-NTA resin resuspended in lysis buffer was added to the soluble lysate fraction and binding was conducted with constant mixing for 1 hour at 4°C. The mixture was then loaded into a gravity flow column and the non-binding material was allowed to flow through. The Ni-NTA matrix was washed 3 times with 25 ml of wash buffer (50 mM Na₂HPO₄, pH 6.0, 300 mM NaCl, 10 mM β-mercaptoethanol) and bound material was eluted in 25 ml of elution buffer (50 mM Na₂HPO₄, pH 5.0, 300 mM NaCl, 10mM β-mercaptoethanol). The eluted material was then dialyzed against 3 changes of PBS, sterile filtered and stored at -20°C. The purified recombinant protein was shown by SDS-PAGE analysis to be free of any significant amount of *E. coli* protein. A small number of bands of lower molecular weight were assumed to be proteolytic products of the *L. major* antigen based on their reactivity by western blot analysis. A high titre polyclonal antisera against M15 was generated in rabbits by repeated subcutaneous injection of recombinant protein. Western blot analysis of lysates from *L. major* promastigotes and amastigotes using this antisera indicated that the protein is constitutively expressed throughout the parasite lifecycle.

EXAMPLE 2

PREPARATION OF LDP23

This Example illustrates the preparation of a *Leishmania* antigen Ldp23, having the sequence provided in SEQ ID NO:4.

A. Purification of MHC Class II-associated Peptides from P388D1 Macrophages Infected with *L. donovani*

To ascertain that *in vitro* infection of macrophages would load their MHC class II molecules with parasite peptides, initial experiments were carried out to test the ability of *L. donovani*-infected macrophage cell line P388D1 to present parasite antigens to *L. donovani* specific T-cells. This macrophage cell line was chosen because it has the same H-2 haplotype as the BALB/c mouse, which is a strain of mouse

moderately susceptible to *L. donovani* infection and selected to conduct the *in vivo* experiments. Using a proportion of 3-5 parasites per cell and an initial incubation at room temperature for 4-6 hours follows by 37°C for 24-48 hours, close to 90% of the macrophages were infected. The level of MHC class II molecule expression, as
5 determined by FACS analysis, indicated that infection did not cause an effect on the levels of MHC class II expression when compared to non-infected control cells.

To test the ability of the *L. donovani*-infected P388D1 cells to present parasite antigens, macrophages were infected as indicated above and incubated at 26°C for 6 hours, and then as 37°C for either 24, 48 or 72 hours. At each of these time points
10 the non-adherent cells and free parasites were washed out and the adherent cells were mechanically dislodged, washed and fixed with paraformaldehyde. These cells were then used as antigen presenting cells (APCs) for purified lymph node T-cells from BALB/c mice immunized with *L. donovani* promastigotes. To generate these anti-*L. donovani* specific T-cells, BALB/c mice (H-2^d) of both sexes (The Jackson Laboratory,
15 Bar Harbor, ME) were immunized at 8 to 14 weeks of age in the rear foot pad with 5-10 x 10⁶ *L. donovani* promastigotes emulsified in complete Freund's adjuvant (CFA) (Difco Laboratories, Madison, MI) as described in Rodrigues et al., *Parasite Immunol.* 14:49 (1992). The draining lymph nodes were excised 8 days after the immunization and T-cells were purified in an anti-mouse Ig column to remove the B cells, as
20 described in Bunn-Moreno and Campos-Neto, *J. Immunol.* 127:427 (1981), followed by a passage through a Sephadex G10 column to remove the macrophages.

Stimulation index was calculated by dividing the cpm obtained for the cells cultured in the presence of infected P388D1 macrophages by the cpm obtained for the cells cultured in the presence of non-infected macrophages, but subjected to the
25 same conditions as the infected macrophages. The results shown Figure 1 indicate that *L. donovani*-infected P388D1 macrophage process parasite antigens and that optimal presentation occurs after 48 hours of infection. No stimulation of the T-cells by the non-infected macrophages was observed.

To isolate the MHC class II associated *L. donovani* peptides, P388D1
30 macrophages were infected with *L. donovani* promastigotes for an initial incubation of 6

hours at room temperature. The cultures were then transferred to 37°C for the remainder of the 48 hour incubation period. At a ratio of 3-5 parasites per macrophage nearly 90% of the macrophages were infected after 24 hours of incubation at 37°C.

The MHC class II molecules were then affinity-purified. Approximately
5 1.5×10^{10} *L. donovani*-infected or an equal number of non-infected P388D1 macrophages were used for each purification. The cells were harvested, washed with PBS and incubated for 30 minutes in cold lysis buffer (PBS, 1% Nonidet P40, 25mM iodoacetamide, 0.04% sodium azide, 1mM aprotinin and 1mM PMSF). The insoluble material was removed by centrifugation at 40,000g for 1 hour and the supernatant was
10 recycled overnight at 4°C over a 5ml anti-MHC class II molecules (H-2^d) Sepharose column (Protein G Sepharose column to which the monoclonal antibody MK-D6 has been bound). Culture supernatants of MK-D6 hybridoma cells (American Type Culture Collection, Rockville, MD) were employed as the source for anti-MHC class II (H-2^d) monoclonal antibody. The column was washed with 50ml of lysis buffer and then with
15 50ml of PBS containing 0.5% octyl glucopyranoside detergent. Bound molecules were eluted from the column with 1M acetic acid in 0.2% NaCl. The MHC/peptide molecules were separated from the IgG (MK-D6 monoclonal antibody) using a Centricon 100 filter unit (Amicon Division, W.R. Grace & Co., Beverly, MA). The peptides were then dissociated from the class II molecules by the addition of acetic acid
20 to 2.5M, followed by separation using a Centricon 10 filter unit. The resulting peptide preparation, present in the low molecular weight sample, was then dried using a speed vac concentrator (Savant Instrument Inc., Farmingdale, NY).

The peptides were redissolved in 200µl of 0.05% TFA and separated by reverse-phase high performance liquid chromatography (RP-HPLC) using a 2.1mm x
25 25cm Vydac C-18 column at a flow rate of 0.15ml/min employing a 1 to 30% acetonitrile gradient (60 min) followed by a 30 to 60% gradient (30 min) and then a 60 to 80% gradient (90-110 min). Non-infected P388D1 cells were similarly processed to serve as background control for endogenous MHC class II associated peptides. Figure 2 shows a representative experiment; four distinct peaks which are present only in the

material isolated from infected macrophages (panel B), and not in the material isolated from uninfected macrophages (panel A) are indicated.

Out of three independent peptide extractions, twenty five distinct HPLC peptide peaks were isolated from *L. donovani*-infected macrophages and were subjected to protein sequence analysis using automated Edman degradation on an Applied Biosystems-477 gas-phase protein sequencer. Protein sequence and amino acid analysis were performed by the W.M. Keck Foundation, Biotechnology Resource Laboratory, Yale University, New Haven, CT. In practically all determinations, no assignment could be made for the first position. Also, in most cases the definition of the amino acid residues of the 10-15 positions was based on the quantitative dominance of one residue over others. Using this approach, the sequences obtained for several peptides showed the presence of 3-6 different residues in many of the 10-15 sequence cycles analyzed for each determination, reflecting a mixture of peptides. In addition, sequences could not be obtained for some peaks because the peptides were blocked. Notwithstanding, three peptides sequences were determined. Amino-acid sequences were searched for identity with proteins in the GenBank database using the GENPETP, PIR and SWISSPROT programs. The sequence data base analysis revealed that one of the peptides was highly homologous to glyceraldehyde-3-phosphate dehydrogenase of various species. Another peptide had homology with elongation factor of several species, including *Leishmania*. The third sequence was not clearly related to any known proteins, and is shown below:

XQXPQ(L/K)VFDEXX (SEQ ID NO:11).

B. Cloning and Sequencing of the Ldp23 Gene

In order to retrieve the *L. donovani* protein that was processed into a peptide associated with the MHC class II molecules of infected macrophages, the peptide sequence of uncertain origin was chosen to guide the strategy for cloning the corresponding parasite gene. A DNA fragment was initially amplified from *L. donovani* promastigote cDNA by PCR. The sense primer was a peptide derived oligonucleotide (5' > GGAATTCCCCCInCAGCTInGTInTTCGAC < 3') (SEQ ID NO:12) containing an *EcoRI* restriction endonuclease site (underlined). The bases were

selected following the preferential codon usage of *L. donovani*, as described in Langford et al., *Exp. Parasitol.* 74:360 (1992). Inosine was used for the residues of positions 4, 6 and 7 because of the low codon usage assurance for the corresponding amino acids. In addition, the carboxyl-terminal L-glutamic acid was not included for the design of the primer. The antisense primer was a poly-thymidine oligonucleotide (oligo dT, downstream primer) containing a *Xho*I restriction endonuclease site.

The gene fragment was amplified from a *L. donovani* promastigote cDNA preparation using the following reaction conditions: one cycle of 3 min at 94°C immediately followed by 35 cycles of 1 min at 94°C, 1 min at 45°C and 1 min at 72°C. The *L. donovani* cDNA was prepared from 5×10^7 washed promastigote forms harvested at the log growth phase (3 days culture). The cDNA was obtained using an Invitrogen cDNA cycle™ kit (Invitrogen Co., San Diego, CA). Oligonucleotide primers were synthesized by the DNA Synthesis Laboratory, Department of Pathology, Yale University School of Medicine.

The PCR products were analyzed by gel electrophoresis. Only one band of approximately 300 bp was obtained. This fragment was cloned and its sequence confirmed the sequence of the peptide-based primer including the glutamic acid codon, deliberately not included in the primer sequence.

The PCR amplified gene fragment was ligated into the pCR™ vector using the TA cloning system (Invitrogen Co., San Diego, CA). Transformants were selected in LB medium containing 100µg/ml ampicillin and the plasmid DNA was isolated using the Wizard™ Minipreps DNA purification kit (Promega Co., Madison, WI). Insert DNA was released with the restriction enzymes *Eco*RI and *Xho*I (New England Biolabs, Beverly, MA), purified from an agarose gel electrophoresis and labeled with 32 P using a random priming method (Megaprime Labeling Kit, Amersham Life Science, Buckinghamshire, England).

This DNA fragment was used as probe to screen a *L. donovani* promastigote cDNA library as described in Skeiky et al., *Infect. Immun.* 62:1643 (1994). An approximately 650 bp cDNA (Ldp23) was excised from the phagemid by *in vivo* excision using the Stratagene protocol. DNA sequencing was performed using the

Sequenase version 2 system (DNA sequencing kit) in the presence or absence of 7-deaza-GTP (United States Biochemical, Cleveland, OH). The sequence is provided as SEQ ID NO:3, and shows complete homology with the original 300 bp PCR fragment. A 525 bp open reading frame containing an ATG codon that follows the last 4 bases of the spliced leader sequence and 3 stop codons adjacent to the poly A tail was identified. This frame also codes the carboxyl terminal sequence (KVFDE) (SEQ ID NO:13) of the purified MHC class II associated peptide. The sequence analysis of the deduced protein sequence revealed one potential glycosylation site (Asn-Cys-Ser) at positions 68-70.

Sequence analysis was performed using the University of Wisconsin Genetics Computer Group Programs and the GenBank and EMBL data bases of protein and DNA sequences. The search for homology of the Ldp23 gene with known sequences revealed no significant homology.

C. Bacterial Expression and Purification of Recombinant Protein

The recombinant *L. donovani* peptide donor protein was produced in *E. coli* transformed with the pGEX 2T expression vector in which the Ldp23 gene was subcloned in frame. PCR was used to subclone the cloned gene in frame into the expression vector pGEX 2T. Primers containing the appropriate restriction site enzymes, initiation and termination codons were: 5' > GGATCCATGGTCAAGTCCCACTACATCTGC <3' (SEQ ID NO:14) for the upstream primer and 5' > GAATTCAGACCGGATAGAAATAAGCCAATGAAA <3' (SEQ ID NO:15) for the downstream primer (restriction sites of *Bam*HI and *Eco*RI are underlined respectively). PCR conditions were as indicated above for the amplification of the original peptide related DNA fragment. The template used was pBluescript plasmid containing the cloned gene from the cDNA library.

Overexpression of the recombinant fusion protein was accomplished by growing the transformed *E. coli* (DH5 α) and inducing the *tac* promoter with 1mM isopropyl- β -thiogalactopyranoside (IPTG) (Stratagene, La Jolla, CA). Cells were collected, centrifuged, and analyzed for the presence of the fusion protein by SDS-PAGE. A glutathione-S-transferase fusion protein of 43-44 kD was produced,

indicating a leishmanial protein of approximately 18 kD, as glutathione-S-transferase (GST) has a MW of 26 kD. However, the fusion protein was very insoluble and therefore could not be purified by affinity chromatography using a glutathione column. The use of low concentrations of detergents like SDS, sarcosyl, deoxycolate, and octyl-
5 glucopyranoside during the extraction steps was efficient to solubilize the protein but unfortunately prevented its binding to the glutathione column. Other maneuvers, such as the growth of the *E. coli* and incubation and induction of the *tac* promoter with IPTG at 33°C, did not improve the protein solubility. However, the purification was achieved by preparative SDS-PAGE. The band was visualized with 0.1M KCl, cut and
10 electroeluted from the gel followed by extensive dialysis against PBS and concentration on Centricon 10 filters.

Approximately 500µg of purified protein was obtained. The purified protein is shown in Figure 3. In panel A, *E. coli* (DH5α) transformed with the expression vector pGEX 2T containing the Ldp23 gene was grown in LB medium and
15 the *tac* promoter was induced with IPTG for 3 hours. The cells were pelleted, resuspended in loading buffer and submitted to SDS-PAGE (10%) under reducing condition. The gel was stained with Coomassie blue. Lane 1 shows the uninduced *E. coli* and lane 2 shows the induced *E. coli*. The arrow indicates the recombinant protein. Panel B shows the protein prepared as in panel A and submitted to a preparative SDS-
20 PAGE. The band corresponding to the overexpressed recombinant fusion protein was identified by KCl, cut out, electroeluted from the gel strip, dialyzed against PBS and submitted to analytical SDS-PAGE (12%). Numbers on the left side indicate the molecular weights of the markers. Attempts to further purify the leishmanial protein by cleaving it out from the fusion protein GST with thrombin were unsuccessful.

25

D. Expression of Ldp23

To ascertain that the Ldp23 peptide is expressed in *Leishmania* organisms, a Northern blot analysis was performed using RNA prepared from different promastigote growth phases (logarithmic and stationary) and from the amastigote form
30 of these parasites.

The RNA was prepared from 2×10^7 parasite cells using the Micro RNA isolation kit (Stratagene, La Jolla, CA) according to the company's recommended instructions. RNA was prepared from *L. donovani* promastigotes (logarithmic growth phase); from *L. major* promastigotes (logarithmic and stationary growth phases); from 5 *L. amazonensis*, both promastigotes (logarithmic and stationary growth phases) and amastigotes purified from CBA/J infected mice; and from *L. pifanoi*, both promastigotes (logarithmic and stationary growth phases) and amastigotes (from axenic culture medium). *L. donovani* (1S strain), *L. amazonensis* (MHOM/BR/77/LTB0016), *L. major* (MHOM/IR/79/LRC-L251) and *L. pifanoi* (MHOM/VE/60/Ltrod) 10 promastigotes were grown and maintained at 26°C in Schneider's medium containing 20% FCS and 50µg/ml gentamicin. The amastigote forms of *L. amazonensis* were obtained by differential centrifugation of a "pus-like" foot pad lesion of a CBA/J mouse infected for 6 months with this parasite. *L. pifanoi* amastigotes were obtained from axenic culture as previously reported by Pan et al., *J. Euk. Microbiol.* 40:213 (1993).

15 The hybridization was carried out at 45°C in the presence of 50% formamide, 5x Denhardt's solution, 0.1% SDS, 100µg/ml single stranded salmon sperm DNA and 5x SSPE using 0.45µm Nytran membrane filters (Schleicher & Schuell, Keene, NH). The probe was the ^{32}P labeled Ldp23 gene.

Figure 4 shows that one single RNA band of 680 bp was observed for all 20 growth phases and forms of all tested *Leishmania*. Within Figure 4, the numbers 1, 2 and 3 refer to RNA obtained from promastigotes at the logarithmic growth phase, promastigotes at the stationary growth phase and amastigote forms, respectively, and the numbers on the left side indicate the molecular weights of the markers in base pairs. This result is consistent with the corresponding gene size (525 bp) and with the 25 molecular weight of the expressed protein and points to the ubiquitous distribution and expression of this gene within the genus *Leishmania*.

E. Induction of Anti-*L. donovani* Antibody Response in Mice and Rabbits by Purified Recombinant Protein

In order to evaluate the immunogenicity of the recombinant leishmanial protein, and to investigate its expression in the parasites, mice and rabbits were immunized with the GST-fusion protein in CFA. BALB/c mice were immunized in the rear foot pad with 5-10 μ g of protein emulsified in CFA. Protein concentration was determined using the Bio-Rad Protein Assay reagent (Bio-Rad Laboratories, Richmond, CA). The mice were boosted 7 days later with 5-10 μ g of protein emulsified in incomplete Freund's adjuvant (IFA) inoculated into the peritoneal cavity. The mice were bled 7 days after the second immunization. New Zealand white rabbits (Millbrook Farm, Amherst, MA) were immunized according to the following protocol: one intramuscular (IM) injection of 25-30 μ g of purified recombinant protein emulsified in CFA into each thigh on day one; one IM injection of 25-30 μ g of purified protein emulsified in IFA into each shoulder on day 7; on day 15, 25-30 μ g of the purified protein in PBS was injected into the subcutaneous tissue. The rabbit was bled 7 days after the last immunization.

Sera were prepared and the anti-*Leishmania* antibody response was measured by Western blot analysis and by FACScan. In both cases *L. donovani* promastigotes were used as antigen. Approximately 2×10^6 *L. donovani* promastigotes were grown in Schneider's medium for 3 days (log phase), were washed with PBS, lysed with SDS-PAGE loading buffer and submitted to electrophoresis under reducing conditions using a 15% polyacrylamide gel. The proteins were transferred onto 0.45 μ Immobilon-P transfer membrane (Millipore Co., Bedford, MA) using a wet-type electroblotter (Mini Trans-Blot Electrophoretic Transfer Cell, Bio Rad Life Science Division, Richmond, CA) for 2 hours at 50 V. The membranes were blocked overnight at room temperature with PBS containing 3% normal goat serum (NGS), 0.2% Tween-20 and 0.05% sodium azide, followed by 3 washes with PBS. The blots were then incubated for 3-4 hours at 4°C with a 1/200 dilution of pre-immune rabbit serum (lane A, Figure 5) or with the same dilution of anti-fusion protein rabbit antiserum (lane B, Figure 5). The sera was previously absorbed 2x with non-viable desiccated *Mycobacterium tuberculosis* H-37 RA (Difco Laboratories, Detroit, MI) and were diluted in PBS containing 1% NGS and 5% powdered non-fat bovine milk (Carnation,

Nestlé Food Company, Glendale, CA). The membranes were then washed with PBS, incubated for 1 hour at room temperature with goat anti-rabbit IgG antibody conjugated with alkaline phosphatase (Promega, Madison, WI), washed once with PBS and 2x with veronal buffer pH 9.4. The reaction was visualized using the substrate mixture 5-bromo-4-chloro-3-indoyl-phosphate and nitroblue tetrazolium (Kirkegaard & Perry Laboratories Inc., Gaithersburg, MD) according to the manufacturer's instructions.

Figure 5 shows that the rabbit anti-recombinant protein antiserum detects a single protein of 23 kDa (Ldp23) in the *Leishmania* crude extract antigen preparation. No bands were observed when an anti-GST antiserum was used (not shown). Moreover, the FACScan analysis (Figure 6) shows that the antibody induced by the recombinant Ldp23 reacts with intact live *L. donovani* promastigotes, thus pointing to a cell surface expression of this molecule on these organisms. The dotted line in Figure 6 shows the indirect immunofluorescence performed using pre-immune mouse serum and the solid line in Figure 6 shows the result obtained with mouse anti-GST-Ldp23 antiserum. Both sera were diluted at 1/100. Parasites were washed with staining buffer and incubated with FITC conjugated goat anti-mouse immunoglobulin antibody. Fluorescence intensity was analyzed by FACScan.

F. Recognition of Recombinant Ldp23 by *Leishmania*-Specific Lymph Node T-cells

To test the responsiveness of T-cells to the Ldp23 protein, two sets of experiments were performed. In the first experiment, lymph node T-cells (10^5 /well) from BALB/c mice immunized with *L. donovani* promastigotes (as described above) were stimulated to proliferate with 2×10^5 Mitomycin C-treated normal mononuclear spleen cells (APC) and pulsed with the purified recombinant fusion protein. Proliferation of T-cells was measured at 72 hours of culture. Values are expressed in Figure 7 as cpm and represent the mean of [3 H]TdR incorporation of triplicate cultures. Background cpm of cells (T cells + APC) cultured in the presence of medium alone was 1291. Figure 7 shows that *Leishmania* specific T-cells proliferate well and in a dose response manner to recombinant Ldp23. No response was observed when purified GST

was added instead of the recombinant fusion protein nor when lymph node T-cells from mice immunized with CFA alone were stimulated to proliferate in the presence of the Leishmanial fusion protein (not shown).

The recognition of the recombinant Ldp23 protein by *Leishmania*-specific T-cells was also tested using two murine models of leishmaniasis, the *L. major* highly susceptible BALB/c mice and the *L. amazonensis* susceptible CBA/J mice as described in Champsi and McMahon-Pratt, *Infect. Immun.* 56:3272 (1988). These models were selected to investigate the cytokine pattern induced by Ldp23. In the mouse model of leishmaniasis, resistance is associated with Th 1 cytokines while susceptibility is linked to Th 2 responses.

Lymph node cells were obtained 3 weeks after the initiation of infection of BALB/c mice with *L. major* and the ability of these cells to recognize the recombinant Ldp23 was measured by proliferation and by the production of the cytokines IFN- γ and IL-4. 2×10^6 cells obtained from the draining popliteal lymph node of infected mice were cultured for 72 hours in the presence of recombinant Ldp23 or *Leishmania* lysate. The levels of IFN- γ and IL-4 in culture supernatants were measured by ELISA as previously described (Chatelain et al., *J. Immunol.* 148:1172 (1992), Curry et al., *J. Immunol. Meth.* 104:137 (1987), and Mossman and Fong, *J. Immunol. Meth.* 116:151 (1989)) using specific anti IFN- γ and IL-4 monoclonal antibodies (PharMingen, San Diego, CA).

Ldp23 did stimulate these cells to proliferate (not shown) and induced a typical Th 1 type of cytokine response as indicated by the production of high levels of IFN- γ (panel A of Figure 8) and no IL-4 (panel B of Figure 8). Stimulation of these cells with a *Leishmania* crude lysate yielded a mixed Th cytokine profile. Exactly the same pattern of cytokine production was obtained from the CBA/J mice infected with *L. amazonensis* (not shown). These results clearly indicate that Ldp23 is a powerful and selective activator of the Th 1 cytokines by mouse cells.

EXAMPLE 3

PREPARATION OF HSP83

This Example illustrates the preparation of a *Leishmania* antigen Hsp83,
5 having the sequence provided in SEQ ID NO:6.

A genomic expression library was constructed with sheared DNA from
L. braziliensis (MHOM/BR/75/M2903) in bacteriophage λ ZAP II (Stratagene, La Jolla,
CA). The expression library was screened with *Escherichia coli* preadsorbed serum
from an *L. braziliensis*-infected individual with ML. Immunoreactive plaques were
10 purified, and the pBSK(-) phagemid was excised by protocols suggested by the
manufacturer. Nested deletions were performed with exonuclease III to generate
overlapping deletions for single-stranded template preparations and sequencing. Single-
stranded templates were isolated following infection with VCSM13 helper phage as
recommended by the manufacturer (Stratagene, La Jolla, CA) and sequenced by the
15 dideoxy chain terminator method or by the *Taq* dye terminator system using the
Applied Biosystems automated sequencer model 373A.

Recombinant antigens produced by these clones were purified from 500
ml of isopropyl- β -D-thiogalactopyranoside (IPTG)-induced cultures as described in
Skeiky et al., *J. Exp. Med.* 176:201-211 (1992). These antigens were then assayed for
20 the ability to stimulate PBMC from *Leishmania*-infected individuals to proliferate and
secrete cytokine. Peripheral blood was obtained from individuals living in an area
(Corte de Pedra, Bahia, Brazil) where *L. braziliensis* is endemic and where
epidemiological, clinical, and immunological studies have been performed for over a
decade, and PBMC were isolated from whole blood by density centrifugation through
25 Ficoll (Winthrop Laboratories, New York, N.Y.). For *in vitro* proliferation assays, 2×10^5
to 4×10^5 cells per well were cultured in complete medium (RPMI 1640
supplemented with gentamicin, 2-mercaptoethanol, L-glutamine, and 10% screened
pooled A+ human serum; Trimar, Hollywood, Calif.) in 96-well flat-bottom plates with
or without 10 μ g of the indicated antigens per ml or 5 μ g of phytohemagglutinin per ml
30 (Sigma Immunochemicals, St. Louis, Mo.) for 5 days. The cells were then pulsed with

1 μ Ci of [3 H]thymidine for the final 18 h of culture. For determination of cytokine production 0.5 to 1 ml of PBMC was cultured at 1×10^6 to 2×10^6 cells per ml with or without the *Leishmania* antigens for 48 and 72 h.

The supernatants and cells were harvested and analyzed for secreted
5 cytokine or cytokine mRNAs. Aliquots of the supernatants were assayed for gamma interferon (IFN- γ), tumor necrosis factor alpha (TNF- α), interleukin-4 (IL-4), and IL-10 as described in Skeiky et al., *J. Exp. Med.* 181:1527-1537 (1995). For cytokine mRNA PCR analysis, total RNA was isolated from PBMC and cDNA was synthesized by using poly(dT) (Pharmacia, Piscataway, NJ) and avian myeloblastosis virus reverse
10 transcriptase. Following normalization to β -actin, diluted cDNA was amplified by PCR using *Taq* polymerase (Perkin-Elmer Cetus, Foster City, CA) with 0.2 μ M concentrations of the respective 5' and 3' external primers in a reaction volume of 50 μ l. The nucleotide sequences of the primary pairs and the PCR conditions used were as described in Skeiky et al., *J. Exp. Med.* 181:1527-1537 (1995). We verified that our
15 PCR conditions were within the semiquantitative range by initially performing serial dilutions of the cDNAs and varying the number of cycles used for PCR. Plasmids containing the human sequences for IL-2, IFN- γ , IL-4, IL-10, and β -actin were digested, and the DNA inserts were purified after separation on 1% agarose gels. Radiolabeled 32 P probes were prepared by the random priming method. PCR products were analyzed
20 by electrophoresis on 1.5% agarose gels, transferred to nylon membranes, and probed with the appropriate 32 P-labeled DNA insert.

A recombinant clone was identified in the above assays which, following sequence comparison of its predicted amino acid sequence with sequences of other proteins, was identified as a *Leishmania braziliensis* homolog of the eukaryotic 83 kD
25 heat shock protein (Lbhsp83). The sequence of the clone is provided in SEQ ID NO:5 and the deduced protein sequence is provided in SEQ ID NO:6. On the basis of the homology, this clone, designated Lbhsp83a, appears to lack the first 47 residues of the full length 703 amino acid residues. Lbhsp83 has an overall homology of 94% (91% identity and 3% conservative substitution), 91% (84% identity and 7% conservative
30 substitution) and 77% (61% identity and 16% conservative substitution) with *L.*

amazonensis hsp83, *T. cruzi* hsp83 and human hsp89, respectively. A second clone (designated Lbhsp83b), which contained the 43 kD C-terminal portion of hsp83 (residues 331 to 703) was also isolated. Figure 19 presents a comparison of the Lbhsp83 sequence with *L. amazonensis* hsp83(Lahsp83), *T. cruzi* hsp83 (Tchsp83) and human hsp89 (Huhsp89).

The results of proliferation assays using Lbhsp83a are shown in Table 1. Cells from all mucosal leishmaniasis (ML) patients proliferated strongly in response to Lbhsp83a, with stimulation indices (SIs) ranging from 19 to 558 (as compared to 20 to 1,634 for parasite lysate). Proliferation of PBMC from cutaneous leishmaniasis (CL) patients was variable and except for levels in two patients (IV and VII), levels were significantly lower than those of ML patients. By comparison, the proliferative responses of individuals with self-healing CL to Lbhsp83a were similar to those of individuals with ML. However, the responses of all six self-healing individuals to Lbhsp83 were consistently higher than those to Lbhsp83b. This suggests that PBMC from self-healing CL patients preferentially recognize one or more T-cell epitopes located within the amino portion of Lbhsp83.

Table 1

In vitro Proliferation of PMBC from *L. braziliensis*-infected Individuals
in Response to Lbhsp83

Group and Patient	Mean [³ H]thymidine incorporation [10 ³ cpm (SD)], SI with:		
	Lysate	Lbhsp83a	Lbhsp83b
ML			
I	41.3, (1.3), 294	32.5, (6.6), 221	46.7, (1.4), 318
II	44.2, (0.5), 104	20, (3.7), 47	36.7, (0.76), 86
III	27.4, (1.5), 150	8.1, (1.7), 44	9.9, (0.32), 54
IV	52.7, (3.3), 138	54.1, (6.2), 142	32.0, (1.3), 84
V	140.6, (7.6), 308	151.8, (57), 333	150.4, (7.9), 331
VI	15.8, (1.8), 20	21.3, (4.4), 28	14.4, (1.3), 19
VII	300.1, (9.4), 1634	102.1, (7.6), 558	41.7, (4.9), 228

Group and Patient	Mean [^3H]thymidine incorporation [10^3 cpm (SD)], SI with:		
	Lysate	Lbhsp83a	Lbhsp83b
CL			
I	0.26, (0.0), 1.5	0.57, (0.3), 3.3	0.43, (0.17), 3.3
II	55.63, (8.6), 218	0.42, (0.0), 1.6	0.8, (0.14), 3.2
III	0.39, (0.5), 4.0	3.4, (0.5), 9	2.6, (0.9), 6.6
IV	19.14, (1.3), 87	7.17, (0.6), 32	5.9, (0.9), 27
V	0.32, (0.2), 3.0	1.47, (0.5), 14	0.3, (0.1), 3.0
VI	0.77, (0.1), 4.7	1.44, (0.2), 9	1.3, (0.6), 8.0
VII	4.01, (1.0), 2.0	60.3, (8.5), 15	66.7, (3.9), 16.6
Self-healing CL			
I	19.7, (4.4), 94	61.3, (4.6), 293	5.0, (2.0), 24
II	0.6, (0.1), 6.5	7.0, (2.0), 79	1.2, (0.8), 13
III	59.6, (7.1), 519	49.4, (3.1), 429	21.4, (3.7), 186
IV	0.2, (0.1), 1.6	13.1, (1.7), 108	0.6, (0.1), 5
V	27.1, (2.0), 225	6.3, (2.6), 52	3.0, (1.5), 25
VI	130.3, (14), 340	28.2, (2.9), 74	7.7, (3.8), 20
Control (uninfected)			
I	0.19, (0.0), 1.4	0.18, (0.0), 1.3	0.40, (0.16), 2.8
II	0.31, (0.1), 1.7	0.19, (0.0), 1.0	0.27, (0.0), 1.5
III	0.44, (0.2), 4.1	0.48, (0.1), 5.0	0.51, (0.2), 5.2
IV	0.4, (0.1), 3.2	0.52, (0.2), 5.1	0.50, (0.1), 5.0

A more detailed analysis of cytokine patterns of PBMC from ML patients was performed by reverse transcriptase PCR. Cytokine mRNAs were evaluated in cells prior to culturing (Figure 9, lanes O) or following culturing in the absence (lanes -) or presence of the indicated antigen for 48 and 72 h. Figure 4A shows the results for five of the six ML patients whose PBMC were analyzed. In about half of the ML patients, noncultured (resting) PBMC had detectable levels of mRNA for IFN- γ , IL-2, and IL-4 but not IL-10. CL patient PBMC, however, had IL-10 mRNA in the resting state in addition to mRNAs for the other cytokines tested (Figure 4B).

Following *in vitro* culture without antigen, the levels of mRNA for IFN- γ , IL-2, and IL-4 in resting cells from ML patients decreased to background levels while IL-10 mRNA levels increased. In contrast, PBMC of most CL patients had stable or increased IL-10

mRNA, while the mRNAs for IL-2, IFN- γ , and IL-4 were reduced to barely detectable levels in the absence of antigen stimulation.

In PBMC of three ML patients, stimulation with lysate resulted in increased expression of mRNA for IFN- γ , IL-2, and IL-4 but not IL-10. By comparison, both Lbhsp83 polypeptides elicited the production of mRNA for IFN- γ and IL-2 from all ML patient PBMC tested. In contrast, profiles of mRNA for IL-10 and IL-4 differed for the two hsp83 polypeptides. Lbhsp83a stimulated the production of IL-10 but not IL-4 mRNA (patients I, II, III, and IV), while Lbhsp83b stimulated the production of IL-4 but not IL-10 mRNA in all six patients.

All CL patients tested responded to both Lbhsp83 polypeptides as well as to the parasite lysate by upregulating the synthesis of mRNAs for IL-2 and IFN- γ , and in two of four patients (I and IV), the level of IL-4 mRNA also increased, indicating stimulation of both Th1 and Th2 cytokines. Interestingly and as in the case of ML patient uncultured PBMC which did not have detectable levels of IL-10 mRNA, Lbhsp83a and not Lbhsp83b stimulated PBMC from one CL patient (IV) to synthesize IL-10 mRNA. However, in the other three patients (I, II, and III) with resting levels of IL-10 mRNA, both rLbhsp83 polypeptides as well as the parasite lysate downregulated the expression of IL-10 mRNA.

PBMC supernatants were also assayed for the presence of secreted IFN- γ , TNF- α , IL-4, and IL-10. Cells from all ML and self-healing CL patients (seven and six patients, respectively) and from four of seven CL patients were analyzed for secreted IFN- γ following stimulation with both rLbhsp83 polypeptides, parasite lysate and Lbhsp70, an *L. braziliensis* protein homologous to the eukaryotic 70 kD heat shock protein (Figure 10A). In general, rLbhsp83a stimulated patient PBMC to secrete higher levels of IFN- γ than did rLbhsp83b (0.2 to 36 and 0.13 to 28 ng/ml, respectively). The presence of secreted IFN- γ correlated well with the corresponding mRNA detected by PCR

PBMC from four of five ML patients (I, II, V, and VII) had supernatant TNF- α levels (0.8 to 2.2 ng/ml) higher than those detected in cultures of PBMC from uninfected controls following stimulation with parasite lysate (Figure 10B). Similarly,

the same PBMC were stimulated by rLbhsp83 to produce levels of TNF- α in supernatant ranging from 0.61 to 2.9 ng/ml. Compared with those of uninfected controls, PBMC from three (I, V, and VI), five (I, II, IV, V, and VI), and two (II and V) of six individuals analyzed produced higher levels of TNF- α in response to parasite lysate, rLbhsp83a, and rLbhsp83b, respectively. The levels of TNF- α produced by PBMC from CL patients in response to parasite lysate were comparable to those produced by uninfected controls. However, rLbhsp83 stimulated TNF- α production in the PBMC of two of these patients. rLbhsp83a stimulated higher levels of TNF- α production than did rLbhsp83b. In the absence of antigen stimulation, only PBMC from ML patients (five of six) produced detectable levels of supernatant TNF- α (60 to 190 pg/ml).

In agreement with the IL-10 mRNA, IL-10 was detected by ELISA in the antigen-stimulated PMBC culture supernatants from ML and CL patients. The levels (49 to 190 pg) were significantly higher (up to 10-fold) following stimulation with rLbhsp83a compared with those after parallel stimulation of the same cells with rLbhsp83b (Figure 11). Parasite lysate also stimulated PMBC from some of the patients to produce IL-10. Although rLbhsp83 stimulated PMBC from uninfected individuals to produce IL-10, with one exception, the levels were lower than those observed with patient PMBC. IL-4 was not detected in any of the supernatants analyzed. Therefore, the level of any secreted IL-4 is below the detection limit of the ELISA employed (50 pg/ml). Taken together, the results demonstrate that a predominant Th1-type cytokine profile is associated with PMBC from *L. braziliensis*-infected individuals following stimulation with rLbhsp83 polypeptides.

To determine the correlation between the observed T-cell responses and antibody production to Lbhsp83, we compared the antibody (immunoglobulin G) reactivities to Lbhsp83 in sera from the three patient groups (Figure 12). The ELISA reactivities of ML patient sera with rLbhsp83a were comparable to those observed with parasite lysate, and in general, there was a direct correlation between ML patient anti-Lbhsp83 antibody titer and T-cell proliferation. Of 23 serum samples from ML patients analyzed, 22 were positive (~96%) with absorbance values of 0.20 to >3.0. Eleven of

the ML patient serum samples had optical density values that were >1 . In general, CL patients had significantly lower anti-Lbhsp83 antibody titers ($\bar{x} = 0.74$; standard error of the mean [SEM] = 0.1) compared to those of ML patients. Therefore, ML and CL patient anti-rhsp83 antibody titers correlated with their respective T-cell proliferative responses. Anti-rLbhsp83 antibody titers were significantly higher in patients with ML ($\bar{x} = 1.5$; SEM = 0.2) than in self-healing CL patients ($\bar{x} = 0.35$; SEM = 0.056), although their T-cell proliferative responses were similar. In fact, anti-Lbhsp83 antibody titers in serum from self-healing CL patients were comparable to those from uninfected controls ($\bar{x} = 0.24$; SEM = 0.028). By using 2 standard deviations greater than the mean absorbance value of uninfected control (0.484) as a criterion for positive reactivity to Lbhsp83, eight of nine of the self-healing patient serum samples tested were negative.

EXAMPLE 4

PREPARATION OF CLONES ENCODING LT-210

This Example illustrates the preparation of clones encoding portions of the *Leishmania* antigen Lt-210, and which has the sequence provided in SEQ ID NO:8.

An expression library was constructed from *L. tropica* (MHOM/SA/91/WR1063C) genomic DNA. The DNA was isolated by solubilizing *L. tropica* promastigotes in 10mM Tris-HCl, pH 8.3, 50mM EDTA, 1% SDS and treating with 100 μ g/ml RNaseA and 100 μ g/ml proteinase K. The sample was then sequentially extracted with an equal volume of phenol, phenol: chloroform (1:1), and Chloroform. DNA was precipitated by adding 0.1 volume of 3M sodium acetate (pH 5.2) and 2.5 volume 95% ethanol. The precipitate was resuspended in 10 μ M Tris, 1mM EDTA. DNA was sheared by passage through a 30-gauge needle to a size range of 2-6 kilobase, and was repaired by incubation with DNA polI in the presence of 100 μ M each dATP, dCTP, dGTP, and dTTP. *EcoRI* adapters were ligated to the DNA

fragments. After removal of unligated adapters by passage over a G-25 Sephadex™ column, the fragments were inserted in *EcoRI* cut Lambda ZapII (Stratagene, La Jolla, CA).

Approximately 43,000 pfu were plated and screened with sera isolated from viscerotropic leishmaniasis (VTL) patients. Sera from VTL patients were received from Drs. M. Grogil and A. Magill. The VTL patient group included eight individuals from whom parasites were isolated and cultured, seven of which had confirmed infection with *L. tropica*. Four other patients were culture negative, but were still considered to be infected based on either PCR analysis or a positive monoclonal antibody smear (Dr. Max Grogil, personal communication). Serum samples from the 11 infected patients were pooled and anti-*E. coli* reactivity removed by affinity chromatography (Sambrook et al., *supra*, p. 12.27-12.28). Lambda phage expressing reactive proteins were detected after antibody binding by protein A-horseradish peroxidase and ABTS substrate.

Three clones, Lt-1, Lt-2, and Lt-3, containing a portion of the Lt-210 gene were identified and purified. The clones ranged in size from 1.4 to 3.3 kb and encoded polypeptides of 75 kD, 70 kD, and 120 kD, respectively. These three clones contain partial sequences of the Lt-210 gene. Lt-1 and Lt-2 are overlapping clones and were chosen for further study.

The DNA sequences of Lt-1 and Lt-2 were determined. Exonuclease III digestion was used to create overlapping deletions of the clones (Heinikoff, *Gene* 28:351-359, 1984). Single strand template was prepared and the sequence determined with Applied Biosystems Automated Sequencer model 373A or by Sanger dideoxy sequencing. The sequence on both strands of the coding portion of Lt-1 clone was determined. The partial sequence of one strand of Lt-2 clone was determined.

SEQ ID NO:7 presents the DNA sequence of Lt-1, and SEQ ID NO:8 provides the predicted amino acid sequence of the open reading frame. The DNA sequence of the coding portion of the Lt-1 clone includes a repeated nucleotide sequence at the 5' portion of the clone containing eight copies of a 99 bp repeat, three copies of a 60 bp repeat unit, which is part of the larger 99 bp repeat, and 800 bp of

non-repeat sequence. The deduced amino acid sequence of the 99 bp repeat contains limited degeneracies. The mass of the predicted recombinant protein is 67,060 Daltons. A database search of PIR with the predicted amino acid sequence of the open reading frame yielded no significant homology to previously submitted sequences. Predicted
5 secondary structure of the repeat portion of the clone is entirely α -helical.

Sequence analysis of Lt-2 revealed that the 3' portion of the clone consisted of a mixture of 60 and 99 bp repeats that were identical, excepting occasional degeneracies, to the 60 and 99 bp repeats observed in Lt-1. Collectively, the sequencing data suggest that Lt-1 and Lt-2 are different portions of the same gene, Lt-2
10 being upstream of Lt-1, with possibly a small overlap.

Hybridization analysis confirmed that rLt-2 and rLt-1 contain overlapping sequences. Genomic DNAs of various *Leishmania* species were restricted with a variety of enzymes, separated by agarose gel electrophoresis, and blotted on Nytran membrane filter (Schleicher & Schuell, Keene, NH). Inserts from rLt-1 and rLt-
15 2 were labeled with ^{32}P -CTP by reverse transcriptase from random oligonucleotide primers and used as probes after separation from unincorporated nucleotides on a Sephadex G-50 column. Hybridizations using the rLt-1 or the rLt-2 probe are performed in 0.2M NaH_2PO_4 /3.6 M NaCl at 65°C, whereas hybridization using the rLt-1r probe is performed in 0.2 M NaH_2PO_4 /3.6 M NaCl/0.2 M EDTA at 60°C overnight.
20 Filters are washed in 0.075 M NaCl/0.0075 M sodium citrate pH 7.0 (0.15 M NaCl/0.0150 M sodium citrate for the Lt-1r probe), plus 0.5% SDS at the same temperature as hybridization.

Genomic DNA from a number of *Leishmania* species including *L. tropica* were analyzed by Southern blots as described above using the Lt-1, Lt-2, and
25 Lt-1r inserts separately as probes. Collectively, various digests of *L. tropica* DNA indicate that this gene has a low copy number. A similar, overlapping pattern was observed using either the Lt-1 or Lt-2 insert as a probe, consistent with the premise that these two clones contain sequences near or overlapping one another. In addition, sequences hybridizing with these clones are present in other *Leishmania* species.

L. tropica isolates have limited heterogeneity. Southern analyses of digested genomic DNA from four *L. tropica* parasite strains isolated from VTL patients and three *L. tropica* parasite strains isolated from CL cases (two human, one canine) were performed. The Lt-lr insert described below was labeled and used as a probe. The
5 seven different *L. tropica* isolates yielded similar intensities and restriction patterns, with only a single restriction fragment length polymorphism among the isolates. These data, along with Southern analyses with additional enzymes, indicate limited heterogeneity in this region among the *L. tropica* isolates.

The recombinant proteins of Lt-1 and Lt-2 were expressed and purified.
10 The nested deletion set of Lt-1 formed for sequencing included a clone referred to as Lt-lr, which contains one and one-third repeats. This polypeptide was also expressed and purified. *In vivo* excision of the pBluescript SK⁻ phagemid from Lambda Zap II was performed according to the manufacturer's protocol. Phagemid virus particles were used to infect *E. coli* XL-1 Blue. Production of protein was induced by the addition of
15 IPTG. Protein was recovered by first lysing pellets of induced bacteria in buffer (LB, 50 mM Tris-HCl, pH 8.0, 100 mM NaCl, 10 mM EDTA) using a combination of lysozyme (750µg/mL) and sonication. rLt-1, rLt-2, and rLt-lr, were recovered from the inclusion bodies after solubilization in 8M urea (rLt-1 and rLt-2) or 4M urea (rLt-lr). Proteins rLt-1 and rLt-2 were enriched and separated by precipitation with 25%-40%
20 ammonium sulfate and rLt-lr was enriched by precipitation with 10%-25% ammonium sulfate. The proteins were further purified by preparative gel electrophoresis in 10% SDS-PAGE. Recombinant proteins were eluted from the gels and dialyzed in phosphate-buffered saline (PBS). Concentration was measured by the Pierce (Rockford, IL) BCA assay, and purity assessed by Coomassie blue staining after SDS-
25 PAGE.

EXAMPLE 5

PREPARATION OF LbEIF4A

This example illustrates the molecular cloning of a DNA sequence
5 encoding the *L. braziliensis* ribosomal antigen LbEIF4A.

A genomic expression library was constructed with sheared DNA from
L. braziliensis (MHOM/BR/75/M2903) in bacteriophage λ ZAPII (Stratagene, La Jolla,
CA). The expression library was screened with *E. coli*-preadsorbed patient sera from an
L. braziliensis-infected individual with mucosal leishmaniasis. Plaques containing
10 immunoreactive recombinant antigens were purified, and the pBSK(-) phagemid
excised using the manufacturer's protocols. Nested deletions were performed with
Exonuclease III to generate overlapping deletions for single stranded template
preparations and sequencing. Single stranded templates were isolated following
infection with VCSM13 helper phage as recommended by the manufacturer (Stratagene,
15 La Jolla, CA) and sequenced by the dideoxy chain terminator method or by the Taq dye
terminator system using the Applied Biosystems Automated Sequencer Model 373A.

The immunoreactive recombinant antigens were then analyzed in patient
T-cell assays for their ability to stimulate a proliferative and cytokine production, as
described in Examples 7 and 8 below.

20 A recombinant clone was identified in the above assays which, following
sequence comparison of its predicted amino acid sequence with sequences of other
proteins, was identified as a *Leishmania braziliensis* homolog of the eukaryotic
initiation factor 4A (eIF4A). The isolated clone (pLeIF.1) lacked the first 48 amino
acid residues (144 nucleotides) of the full length protein sequence. The pLeIF.1 insert
25 was subsequently used to isolate the full length genomic sequence.

SEQ ID NO:9 shows the entire nucleotide sequence of the full-length
LbEIF4A polypeptide. The open reading frame (nucleotides 115 to 1323) encodes a 403
amino acid protein with a predicted molecular weight of 45.3 kD. A comparison of the
predicted protein sequence of LbEIF4A with the homologous proteins from tobacco
30 (TeIF4A), mouse (MeIF4A), and yeast (YeIF4A) shows extensive sequence homology,

with the first 20-30 amino acids being the most variable. The lengths (403, 413, 407, and 395 amino acids), molecular weights (45.3, 46.8, 46.4, and 44.7 kDa), and isoelectric points (5.9, 5.4, 5.5, and 4.9) of LbeIF4A, TeIF4A, MeIF4A and YeIF4A, respectively, are similar. LbeIF4A shows an overall homology of 75.5% (57% identity, 18.5% conservative substitution) with TeIF4A, 68.6% (50% identity, 18.6% conservative substitution) with MeIF4A and 67.2% (47.6% identity, 19.6% conservative substitution) with YeIF4A.

10

EXAMPLE 6

PREPARATION OF SOLUBLE *LEISHMANIA* ANTIGENS

This Example illustrates the preparation of soluble *Leishmania* antigens from an *L. major* culture supernatant. *L. major* promastigotes were grown to late log phase in complex medium with serum until they reached a density of $2-3 \times 10^7$ viable organisms per mL of medium. The organisms were thoroughly washed to remove medium components and resuspended at $2-3 \times 10^7$ viable organisms per mL of defined serum-free medium consisting of equal parts RPMI 1640 and medium 199, both from Gibco BRL, Gaithersburg, MD. After 8-12 hours, the supernatant was removed, concentrated 10 fold and dialyzed against phosphate-buffered saline for 24 hours. Protein concentration was then determined and the presence of at least eight different antigens confirmed by SDS-PAGE. This mixture is referred to herein as "soluble *Leishmania* antigens."

25

EXAMPLE 7COMPARISON OF INTERLEUKIN-4 AND INTERFERON- γ PRODUCTION
STIMULATED BY *LEISHMANIA* ANTIGENS

5 This Example illustrates the immunogenic properties of the antigens prepared according to Examples 1, 2, 5 and 6, as determined by their ability to stimulate IL-4 and IFN- γ in lymph node cultures from infected mice and in human PBMC preparations. Lymph node cultures for use in these studies were prepared from *L. major*-infected BALB/c mice 10 days after infection, as described in Example 2.

10 PBMC were prepared using peripheral blood obtained from individuals with cured *L. donovani* infections who were immunologically responsive to *Leishmania*. Diagnosis of the patients was made by clinical findings associated with at least one of the following: isolation of parasite from lesions, a positive skin test with *Leishmania* lysate or a positive serological test. Uninfected individuals were identified based on a lack of

15 clinical signs or symptoms, a lack of history of exposure or travel to endemic areas, and the absence of a serological or cellular response to *Leishmania* antigens. Peripheral blood was collected and PBMC isolated by density centrifugation through Ficoll™ (Winthrop Laboratories, New York).

Culture supernatants were assayed for the levels of secreted IL-4 and

20 IFN- γ . IFN- γ was quantitated by a double sandwich ELISA using mouse anti-human IFN- γ mAb (Chemicon, Temucula, CA) and polyclonal rabbit anti-human IFN- γ serum. Human rIFN- γ (Genentech Inc., San Francisco, CA) was used to generate a standard curve. IL-4 was quantitated in supernatants by a double sandwich ELISA using a mouse anti-human IL-4 mAb (M1) and a polyclonal rabbit anti-human IL-4 sera (P3).

25 Human IL-4 (Immunex Corp., Seattle, WA) was used to generate a standard curve ranging from 50 pg/ml to 1 ng/ml.

Figures 13A and 13B, illustrate the mean level of secreted IL-4 and IFN- γ , respectively, 72 hours after addition of 10 μ g/mL of each of the following antigens to a lymph node culture prepared as described above: soluble *Leishmania* antigen (*i.e.*, an

30 extract prepared from ruptured promastigotes which contains membrane and internal

antigens (SLA)), Ldp23, LbeIF4A (LeIF), Lbhsp83, M15 and LmeIF (the *L. major* homolog of LbeIF4A). The levels of secreted IL-4 and IFN- γ in medium alone (*i.e.*, unstimulated) are also shown. While SLA elicits a predominantly Th2 response from lymph node cells of Leishmania-infected mice, Ldp23, LbeIF4A, Lbhsp83 and M15
5 elicited relatively little IL-4 and large amounts of IFN- γ , consistent with a Th1 response profile.

Figure 14 shows the level of secreted IFN- γ in culture filtrate from infected and uninfected human PBMC preparations 72 hours after addition of 10 μ g/mL *L. major* lysate, M15 or L-Rack, an immunodominant leishmanial antigen in murine
10 leishmaniasis. Similarly, Figure 15 illustrates the level of secreted IFN- γ in culture filtrate from infected and uninfected human PBMC preparations 72 hours after addition of 10 μ g/mL *L. major* lysate, soluble *Leishmania* antigens (prepared as described in Example 6) or L-Rack. These results indicate that M15 and soluble *Leishmania* antigens, but not L-Rack, are potent stimulators of IFN- γ production in patient PBMC,
15 but not in PBMC obtained from uninfected individuals. Thus, M15 and soluble *Leishmania* antigens elicit a dominant Th1 cytokine profile in both mice and humans infected with *Leishmania*.

20

EXAMPLE 8

COMPARISON OF PROLIFERATION STIMULATED BY *LEISHMANIA* ANTIGENS

This Example illustrates the immunogenic properties of the antigens prepared according to Examples 1, 2, 5 and 6, as determined by their ability to stimulate
25 proliferation in lymph node cultures from infected mice and in human PBMC preparations.

For *in vitro* proliferation assays, 2 - 4 x 10⁵ cells/well were cultured in complete medium (RPMI 1640 supplemented with gentamycin, 2-ME, L-glutamine, and 10% screened pooled A+ human serum; Trimar, Hollywood, CA) in 96-well flat
30 bottom plates with or without 10 μ g/ml of the indicated antigens or 5 μ g/ml PHA

(Sigma Immunochemicals, St. Louis, MO) for five days. The cells were then pulsed with 1 μ Ci of [3 H] thymidine for the final 18 hours of culture.

Figure 16 illustrates the proliferation observed after addition of 10 μ g/mL or 20 μ g/mL of each of the following antigens to a lymph node culture prepared as described in Example 7: SLA, Ldp23, LbeIF4A, Lbhsp83, and M15. The level of proliferation without the addition of antigen is also shown. Data are represented as mean cpm. These results demonstrate that a variety of leishmanial antigens are capable of stimulatory lymph node cell proliferation from *Leishmania*-infected mice.

Figures 17 and 18 illustrate the proliferation observed in human PBMC preparations from *Leishmania*-immune and uninfected individuals following the addition of 10 μ g/mL M15 and soluble *Leishmania* antigens, respectively. These values are compared to the proliferation observed following the addition of culture medium, *L. major* lysate or L-Rack. The results show that M15 and soluble *Leishmania* antigens stimulate proliferation in *Leishmania*-immune PBMC, but not in PBMC obtained from uninfected individuals, demonstrating that M15 and soluble antigens (but not L-Rack) are recognized by PBMC from individuals immune to *Leishmania* due to a previous infection.

EXAMPLE 9

PREPARATION OF LMSP1A AND LMSP9A

This Example illustrates the preparation of two soluble *Leishmania* antigens, Lmsp1a and Lmsp9a.

A. Purification of Lmsp1a and Lmsp9a from a mixture of soluble *L. major* antigens

A high titer rabbit sera was raised against *L. major* soluble antigens, prepared as described above in Example 6. Specifically, a New Zealand white rabbit was immunized subcutaneously at multiple sites with 180 μ g of *L. major* soluble antigens in a suspension containing 100 μ g muramyl dipeptide and 50 % incomplete

Freund's adjuvant. Six weeks later the rabbit was given a subcutaneous boost of 100 µg of the same soluble antigen preparation in incomplete Freund's adjuvant. This was followed by two intravenous boosts spaced two weeks apart, each with 100 µg of the soluble antigen preparation. Sera was collected from the rabbit 11 days after the final
5 boost.

Anti *E. coli* antibody reactivities were removed from the rabbit sera by pre-adsorbing on nitrocellulose filters containing lysed *E. coli*. Adsorbed sera were evaluated by Western blot analysis using 10 µg *Leishmania* promastigote lysate (lane 1) and 1 µg soluble *L. major* antigen mixture (lane 2). As shown in Figure 20, the rabbit
10 sera was found to be reactive with seven dominant antigens of the soluble *L. major* antigen mixture with molecular weights ranging from 18 to >200 kDa. A four times longer exposure of the same blot revealed three additional immunoreactive species with molecular weights less than 18 kDa. The same sera reacted with approximately 10 antigens of the promastigote lysate, but with a pattern significantly different from that
15 observed with the soluble *L. major* antigens (Figure 20). This is suggestive of potential post-translational modification of the same antigen before (intracellular localization) and after secretion/shedding. Such modifications may include cleavage of a leader sequence and/or the addition of carbohydrate molecules to the secreted/shed antigens.

The rabbit sera described above was subsequently used to screen an *L. major* cDNA expression library prepared from *L. major* promastigote RNA using the
20 unidirectional Lambda ZAP (uni-ZAP) kit (Stratagene) according to the manufacturer's protocol. A total of 70,000 pfu of the amplified cDNA library was screened with the rabbit sera at a 1:250 dilution. Nineteen positive clones were confirmed in the tertiary screening. The phagemid were excised and DNA from each of the 19 clones was
25 sequenced using a Perkin Elmer/Applied Biosystems Division automated sequencer Model 373A. All 19 clones were found to represent two distinct sequences, referred to as Lmsp1a and Lmsp9a. The determined cDNA sequences for Lmsp1a and Lmsp9a are provided in SEQ ID NO: 19 and 21, respectively, with the corresponding amino acid sequences being provided in SEQ ID NO: 20 and 22, respectively.

B. Characterization of Lmsp1a and Lmsp9a

Fig. 21 shows the full-length cDNA (SEQ ID NO: 19) and predicted amino acid sequence (SEQ ID NO: 20) for the antigen Lmsp1a. The EcoRI/XhoI insert is 1019 bp long and contains the following features: a) the last 17 nt of the spliced leader sequence characteristic of all trypanosoma nuclearly encoded mRNA; b) 39 nt of 5' untranslated sequence; c) an open reading frame of 453 nt long coding for a 151 deduced amino acid sequence with a predicted molecular mass of 16.641 kDa; and d) 471 nt of 3' untranslated sequence terminating with a poly A tail. The predicted amino acid sequence contains three potential phosphorylation sites at amino acid residues 3, 85 and 102. In addition, Lmsp1a contains an RGD sequence at residue 104, a sequence that may play a role in parasite invasion of the macrophage. RGD sequences have been shown to mediate the binding of various adhesion proteins to their cell surface receptors. There is no obvious leader sequence (secretory signal) at the amino terminal portion suggesting that the protein might be shed or excreted. Lmsp1a appears to be one of the most abundant antigens found in the culture supernatant of live promastigote, since 17 of the 19 clones contain sequences of variable lengths identical to Lmsp1a.

Comparison of the amino acid sequence of Lmsp1a with known sequences using the DNA STAR system (Version 87) revealed that Lmsp1a shares between 65% to 70% homology with the eukaryotic nucleoside diphosphate kinase protein, also referred to in the mouse and human as a tumor metastasis inhibitor gene.

Southern blot analysis of genomic DNA from *L. major* (Friedlander strain) digested with a panel of restriction enzymes (lanes 1 to 7) and six other *Leishmania* species of different geographic locations digested with PstI (lanes 8 to 13) using the full-length cDNA insert of Lmsp1a, demonstrated that Lmsp1a is present in all the species characterized with a high degree of conservation (Fig. 22). This suggests evolutionary significance for the maintenance of Lmsp1a and the existence of homologous species among all the *Leishmania* species.

The remaining two cDNA clones isolated from the soluble *L. major* antigen mixture represent identical sequences (referred to as Lmsp9a; SEQ ID NO: 21), suggesting that the two copies resulted from amplification of the primary library.

Sequencing of the Lmsp9a cDNA revealed that the clone does not contain the full length 5' sequence since it is lacking both the spliced leader and 5' untranslated sequences. The 3' end of the cDNA contains a poly A stretch, as would be expected for a *Leishmania* mRNA. Of the predicted translated sequence (SEQ ID NO: 22), 34 of the
5 201 amino acids (17%) represent cysteine residues. Comparison of the predicted protein sequence with those of known proteins as described above, revealed some homology with other cysteine rich proteins such as the major surface trophozoite antigen of *Giardia lamblia* and furin proteases.

10

EXAMPLE 10

PREPARATION AND CHARACTERIZATION OF MAPS-1A

This Example illustrates the preparation and characterization of the
15 *Leishmania* antigen MAPS-1A (SEQ ID NO: 24).

A pool of sera was obtained from 5 BALB/c mice that had been given a primary immunization and two boosts with crude *L. major* promastigote culture supernatant as described below in Example 12. These mice were subsequently shown to be protected when challenged with a dose of live *L. major* promastigotes generally
20 found to be lethal. The mouse sera thus obtained were used to screen an *L. major* amastigote cDNA expression library prepared as described in Example 1. Several seroreactive clones were isolated and sequenced using a Perkin Elmer/Applied Biosystems Division automated sequencer Model 373A (Foster City, CA).

One of these clones, referred to herein as MAPS-1A, was found to be
25 full-length. Comparison of the cDNA and deduced amino acid sequences for MAPS-1A (SEQ ID Nos: 23 and 24, respectively) with known sequences in the gene bank using the DNA STAR system revealed no significant homologies to known *Leishmania* sequences, although some sequence similarity was found to a group of proteins, known as thiol-specific antioxidants, found in other organisms.

For example, supernatants from suitable host/vector systems which secrete recombinant protein into culture media may be first concentrated using a commercially available filter. Following concentration, the concentrate may be applied to a suitable purification matrix such as an affinity matrix or an ion exchange resin. Finally, one or more reverse phase HPLC steps can be employed to further purify a recombinant protein.

In general, any of a variety of expression vectors known to those of ordinary skill in the art may be employed to express recombinant polypeptides of this invention. Expression may be achieved in any appropriate host cell that has been transformed or transfected with an expression vector containing a DNA molecule that encodes a recombinant polypeptide. Suitable host cells include prokaryotes, yeast and higher eukaryotic cells. Preferably, the host cells employed are *E. coli*, yeast or a mammalian cell line such as COS or CHO. The DNA sequences expressed in this manner may encode naturally occurring antigens, portions of naturally occurring antigens, or other variants thereof. For example, variants of a native antigen may generally be prepared using standard mutagenesis techniques, such as oligonucleotide-directed site-specific mutagenesis, and sections of the DNA sequence may be removed to permit preparation of truncated polypeptides.

In another aspect, the present invention provides epitope repeat sequences, or antigenic epitopes, of a *Leishmania* antigen, together with polypeptides comprising at least two such contiguous antigenic epitopes. As used herein an "epitope" is a portion of an antigen that reacts with sera from *Leishmania*-infected individuals (i.e. an epitope is specifically bound by one or more antibodies present in such sera). As discussed above, epitopes of the antigens described in the present application may be generally identified using techniques well known to those of skill in the art.

In one embodiment, antigenic epitopes of the present invention comprise an amino acid sequence provided in SEQ ID NO:43, 56, 57 or 58. As discussed in more detail below, antigenic epitopes provided herein may be employed in the diagnosis and treatment of *Leishmania* infection, either alone or in combination with other

San Diego, CA, and may generally be used according to the manufacturer's instructions. The level of mRNA encoding one or more specific cytokines may be evaluated by, for example, amplification by polymerase chain reaction (PCR). In general, a polypeptide that is able to induce, in a preparation of about $1-3 \times 10^5$ cells, the
5 production of 30 pg/mL of IL-12, IL-4, IFN- γ , TNF- α or IL-12 p40, or 10 pg/mL of IL-12 p70, is considered able to stimulate production of a cytokine.

Immunogenic portions of the antigens described herein may be prepared and identified using well known techniques, such as those summarized in Paul, Fundamental Immunology, 3rd ed., 243-247 (Raven Press, 1993) and references cited
10 therein. Such techniques include screening polypeptides derived from the native antigen for immunogenic properties using, for example, the representative techniques described herein. An immunogenic portion of a polypeptide is a portion that, within such representative assays, generates an immune response (e.g., proliferation and/or cytokine production) that is substantially similar to that generated by the full length
15 antigen. In other words, an immunogenic portion of an antigen may generate at least about 25%, and preferably at least about 50%, of the response generated by the full length antigen in the model assays described herein.

Portions and other variants of immunogenic *Leishmania* antigens may be generated by synthetic or recombinant means. Synthetic polypeptides having fewer
20 than about 100 amino acids, and generally fewer than about 50 amino acids, may be generated using techniques well known to those of ordinary skill in the art. For example, such polypeptides may be synthesized using any of the commercially available solid-phase techniques, such as the Merrifield solid-phase synthesis method, where amino acids are sequentially added to a growing amino acid chain. See
25 Merrifield, *J. Am. Chem. Soc.* 85:2149-2146, 1963. Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Perkin Elmer/Applied BioSystems Division, Foster City, CA, and may be operated according to the manufacturer's instructions.

Recombinant polypeptides containing portions and/or variants of a
30 native antigen may be readily prepared from a DNA sequence encoding the antigen.

immunizing BALB/c mice (e.g., in the rear foot pad) with *Leishmania* promastigotes emulsified in complete Freund's adjuvant. The draining lymph nodes may be excised following immunization and T-cells may be purified in an anti-mouse Ig column to remove the B cells, followed by a passage through a Sephadex G10 column to remove
5 the macrophages. Similarly, lymph node cells may be isolated from a human following biopsy or surgical removal of a lymph node.

The ability of a polypeptide (e.g., a *Leishmania* antigen or a portion or other variant thereof) to induce a response in PBMC or lymph node cell cultures may be evaluated by contacting the cells with the polypeptide and measuring a suitable
10 response. In general, the amount of polypeptide that is sufficient for the evaluation of about 2×10^5 cells ranges from about 10 ng to about 100 μ g, and preferably is about 1-10 μ g. The incubation of polypeptide with cells is typically performed at 37°C for about 1-3 days. Following incubation with polypeptide, the cells are assayed for an appropriate response. If the response is a proliferative response, any of a variety of
15 techniques well known to those of ordinary skill in the art may be employed. For example, the cells may be exposed to a pulse of radioactive thymidine and the incorporation of label into cellular DNA measured. In general, a polypeptide that results in at least a three fold increase in proliferation above background (i.e., the proliferation observed for cells cultured without polypeptide) is considered to be able to
20 induce proliferation.

Alternatively, the response to be measured may be the secretion of one or more cytokines (such as interferon- γ (IFN- γ), interleukin-4 (IL-4), interleukin-12 (p70 and/or p40), interleukin-2 (IL-2) and/or tumor necrosis factor- α (TNF- α)) or the change in the level of mRNA encoding one or more specific cytokines. In particular,
25 the secretion of interferon- γ , interleukin-2, tumor necrosis factor- α and/or interleukin-12 is indicative of a Th1 response, which is responsible for the protective effect against *Leishmania*. Assays for any of the above cytokines may generally be performed using methods known to those of ordinary skill in the art, such as an enzyme-linked immunosorbent assay (ELISA). Suitable antibodies for use in such assays may be
30 obtained from a variety of sources such as Chemicon, Temucula, CA and PharMingen,

4H6-41 and 8G3-100 may be isolated by means of CD4+ T cell expression cloning as described below. DNA sequences encoding these antigens are provided in SEQ ID NO: 72-79, respectively, with the corresponding predicted amino acid sequences being provided in SEQ ID NO: 80-87. The immunogenic properties of the isolated
5 *Leishmania* antigens may be evaluated using, for example, the representative methods described herein.

Regardless of the method of preparation, the antigens described herein are immunogenic. In other words, the antigens (and immunogenic portions thereof) are capable of eliciting an immune response in cultures of lymph node cells and/or
10 peripheral blood mononuclear cells (PBMC) isolated from presently or previously *Leishmania*-infected individuals. More specifically, the antigens, and immunogenic portions thereof, have the ability to induce T-cell proliferation and/or to elicit a dominantly Th1-type cytokine response (e.g., IL-2, IFN- γ , and/or TNF- α production by T-cells and/or NK cells; and/or IL-12 production by monocytes, macrophages and/or B
15 cells) in cells isolated from presently or previously *Leishmania*-infected individuals. A *Leishmania*-infected individual may be afflicted with a form of leishmaniasis (such as subclinical, cutaneous, mucosal or active visceral) or may be asymptomatic. Such individuals may be identified using methods known to those of ordinary skill in the art. Individuals with leishmaniasis may be identified based on clinical findings associated
20 with at least one of the following: isolation of parasite from lesions, a positive skin test with *Leishmania* lysate or a positive serological test. Asymptomatic individuals are infected individuals who have no signs or symptoms of the disease. Such individuals can be identified based on a positive serological test and/or skin test with *Leishmania* lysate.

25 The term "PBMC," which refers to a preparation of nucleated cells consisting primarily of lymphocytes and monocytes that are present in peripheral blood, encompasses both mixtures of cells and preparations of one or more purified cell types. PBMC may be isolated by methods known to those in the art. For example, PBMC may be isolated by density centrifugation through, for example, Ficoll™ (Winthrop
30 Laboratories, New York). Lymph node cultures may generally be prepared by

medium with serum until they reach a density of $2-3 \times 10^7$ viable organisms per mL of medium. The organisms are thoroughly washed to remove medium components and resuspended at $2-3 \times 10^7$ viable organisms per mL of defined serum-free medium consisting of equal parts RPMI 1640 and medium 199, both from Gibco BRL, Gaithersburg, MD. After 8-12 hours, the supernatant containing soluble *Leishmania* antigens is removed, concentrated 10 fold and dialyzed against phosphate-buffered saline for 24 hours. The presence of at least eight different antigens within the mixture of *Leishmania* antigens may be confirmed using SDS-PAGE (i.e., through the observation of at least 8 different bands). The immunogenic properties of the soluble *Leishmania* antigens may be confirmed by evaluating the ability of the preparation to elicit an immune response in cultures of lymph node cells and/or peripheral blood mononuclear cells (PBMC) isolated from presently or previously *Leishmania*-infected individuals. Such an evaluation may be performed as described below.

Individual antigens present within the mixture of soluble *Leishmania* antigens may be isolated by immunizing mice or rabbits with *Leishmania* culture supernatant, containing soluble antigens, and employing the resultant sera to screen a *Leishmania* cDNA expression library as described in detail below. This procedure may be used to isolate recombinant DNA molecules encoding the *L. major* antigens referred to herein as Lmsp1a, Lmsp9a and MAPS-1A. DNA sequences encoding Lmsp1a, Lmsp9a and MAPS-1A are provided in SEQ ID NO: 19, 21 and 23, respectively, with the corresponding predicted amino acid sequences being presented in SEQ ID NO: 20, 22 and 24, respectively. Similarly, sera from mice or rabbits immunized with *L. major* culture supernatant may be used to screen an *L. major* genomic DNA library. As detailed below, this procedure may be used to isolate DNA molecules encoding the *L. major* antigens referred to herein as LmgSP1, LmgSP3, LmgSP5, LmgSP8, LmgSP9, LmgSP13, LmgSP19, and DNA molecules encoding the *L. chagasi* antigens LcgSP1, LcgSP3, LcgSP4, LcgSP8, and LcgSP10. The DNA sequences encoding these antigens are provided in SEQ ID NO: 29-35 and 44-48, respectively, with the corresponding amino acid sequences being provided in SEQ ID NO: 36-42 and 49-53. The *L. major* antigens referred to herein as 1G6-34, 1E6-44, 4A5-63, 1B11-39, 2A10-37, 4G2-83,

PBMC isolated from *L. braziliensis*-infected patients. Accordingly, Lbhsp83 is an immunogenic *Leishmania* antigen. Regions of Lbhsp83 that are not conserved with the mammalian gene have been found to be particularly potent for T-cell stimulation and antibody binding. Such regions may be identified, for example, by visual inspection of the sequence comparison provided in Figure 19.

This approach may also be used to isolate a DNA molecule encoding a 210 kD immunogenic *L. tropica* antigen, referred to herein as Lt-210. The preparation and characterization of Lt-210, and immunogenic portions thereof (such as Lt-1 and immunogenic repeat and non-repeat sequences), is described in detail in U.S. Patent Application Serial No. 08/511,872, filed August 4, 1995. The sequence of a DNA molecule encoding Lt-1 is provided in SEQ ID NO:7 and the encoded amino acid sequence is presented in SEQ ID NO:8.

The above approach may further be used to isolate a DNA molecule encoding a *L. braziliensis* antigen referred to herein as LbeIF4A. Briefly, such a clone may be isolated by screening a *L. braziliensis* expression library with sera obtained from a patient afflicted with mucosal leishmaniasis, and analyzing the reactive antigens for the ability to stimulate proliferative responses and preferential Th1 cytokine production in PBMC isolated from *Leishmania*-infected patients, as described below. The preparation and characterization of LbeIF4A is described in detail in U.S. Patent Application Serial Nos. 08/454,036 and 08/488,386, which are continuations-in-part of U.S. Patent Application Serial No. 08/232,534, filed April 22, 1994. The sequence of a DNA molecule encoding LbeIF4A is provided in SEQ ID NO:9 and the encoded amino acid sequence is presented in SEQ ID NO:10. Homologs of LbeIF4A, such as that found in *L. major*, may also be isolated using this approach, and are within the scope of the present invention.

Compositions of the present invention may also, or alternatively, contain soluble *Leishmania* antigens. As used herein, "soluble *Leishmania* antigens" refers to a mixture of at least 8 different *Leishmania* antigens that may be isolated from the supernatant of *Leishmania* promastigotes of any species grown for 8-12 hours in protein-free medium. Briefly, the organisms are grown to late log phase in complex

This approach may be used to identify a 23 kD *Leishmania donovani* antigen (referred to herein as Ldp23). The sequence of a DNA molecule encoding Ldp23 is provided in SEQ ID NO:3 and the amino acid sequence of Ldp23 is provided in SEQ ID NO:4. Using the methods described herein, Ldp23 has been shown to induce a Th1 immune response in T-cells prepared from *Leishmania*-infected mice.

Alternatively, a *Leishmania* cDNA or genomic expression library may be screened with serum from a *Leishmania*-infected individual, using techniques well known to those of ordinary skill in the art. DNA molecules encoding reactive antigens may then be used to express the recombinant antigen for purification. The immunogenic properties of the purified *Leishmania* antigens may then be evaluated using, for example the representative methods described herein.

For example, sera from *Leishmania*-infected mice may be used to screen a cDNA library prepared from *Leishmania* amastigotes. Reactive clones may then be expressed and recombinant proteins assayed for the ability to stimulate T-cells or NK cells derived from *Leishmania*-immune individuals (i.e., individuals having evidence of infection, as documented by positive serological reactivity with *Leishmania*-specific antibodies and/or a *Leishmania*-specific DTH response, without clinical symptoms of leishmaniasis). This procedure may be used to obtain a recombinant DNA molecule encoding the *Leishmania* antigen designated M15. The sequence of such a DNA molecule is provided in SEQ ID NO:1, and the amino acid sequence of the encoded protein is provided in SEQ ID NO:2.

A similar approach may be used to isolate a genomic DNA molecule encoding an immunogenic *Leishmania braziliensis* antigen, referred to herein as Lbhsp83. More specifically, a genomic clone encoding Lbhsp83 may be isolated by screening a *L. braziliensis* expression library with sera from a *Leishmania*-infected individual. The DNA encoding Lbhsp83 is homologous to the gene encoding the eukaryotic 83 kD heat shock protein. The sequence of a DNA molecule encoding nearly all of Lbhsp83 is presented in SEQ ID NO:5, and the encoded amino acid sequence is provided in SEQ ID NO:6. Using the methods described below, Lbhsp83 has been found to stimulate proliferation, and a mixed Th1 and Th2 cytokine profile, in

joined by way of a linker sequence (e.g., Gly-Cys-Gly) that does not significantly diminish the immunogenic properties of the component polypeptides.

In general, *Leishmania* antigens having immunogenic properties, and DNA sequences encoding such antigens, may be prepared using any of a variety of procedures from one or more *Leishmania* species including, but not limited to, *L. donovani*, *L. chagasi*, *L. infantum*, *L. major*, *L. amazonensis*, *L. braziliensis*, *L. panamensis*, *L. mexicana*, *L. tropica*, and *L. guyanensis*. Such species are available, for example, from the American Type Culture Collection (ATCC), Rockville, MD. For example, peptides isolated from MHC class II molecules of macrophages infected with a *Leishmania* species may be used to rescue the corresponding *Leishmania* donor antigens. MHC class II molecules are expressed mainly by cells of the immune system, including macrophages. These molecules present peptides, which are usually 13-17 amino acids long, derived from foreign antigens that are degraded in cellular vesicles. The bound peptide antigens are then recognized by CD4 T-cells. Accordingly, foreign peptides isolated from MHC class II molecules of, for example, *Leishmania*-infected murine macrophages may be used to identify immunogenic *Leishmania* proteins.

Briefly, peptides derived from *Leishmania* antigens may be isolated by comparing the reverse phase HPLC profile of peptides extracted from infected macrophages with the profile of peptides extracted from uninfected cells. Peptides giving rise to distinct HPLC peaks unique to infected macrophages may then be sequenced using, for example, Edman chemistry as described in Edman and Berg, *Eur J. Biochem*, 80:116-132 (1967). A DNA fragment corresponding to a portion of a *Leishmania* gene encoding the peptide may then be amplified from a *Leishmania* cDNA library using an oligonucleotide sense primer derived from the peptide sequence and an oligo dT antisense primer. The resulting DNA fragment may then be used as a probe to screen a *Leishmania* library for a full length cDNA or genomic clone that encodes the *Leishmania* antigen. Such screens may generally be performed using techniques well known to those of ordinary skill in the art, such as those described in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY (1989).

asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his.

5 Variants may also (or alternatively) be modified by, for example, the deletion or addition of amino acids that have minimal influence on the immunogenic properties, secondary structure and hydrophobic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification or identification of the polypeptide (e.g., poly-His), or to
10 enhance binding of the polypeptide to a solid support. For example, a polypeptide may be conjugated to an immunoglobulin Fc region.

A nucleotide "variant" is a sequence that differs from the recited nucleotide sequence in having one or more nucleotide deletions, substitutions or additions. Such modifications may be readily introduced using standard mutagenesis
15 techniques, such as oligonucleotide-directed site-specific mutagenesis as taught, for example, by Adelman et al. (*DNA*, 2:183, 1983). Nucleotide variants may be naturally occurring allelic variants, or non-naturally occurring variants. Variant nucleotide sequences preferably exhibit at least about 70%, more preferably at least about 80% and most preferably at least about 90% identity to the recited sequence. Such variant
20 nucleotide sequences will generally hybridize to the recited nucleotide sequence under stringent conditions. As used herein, "stringent conditions" refers to prewashing in a solution of 6X SSC, 0.2% SDS; hybridizing at 65 °C, 6X SSC, 0.2% SDS overnight; followed by two washes of 30 minutes each in 1X SSC, 0.1% SDS at 65 °C and two washes of 30 minutes each in 0.2X SSC, 0.1% SDS at 65 °C.

25 "Polypeptides" as described herein also include combination polypeptides. A "combination polypeptide" is a polypeptide comprising at least one of the above immunogenic portions and one or more additional immunogenic *Leishmania* sequences, which are joined via a peptide linkage into a single amino acid chain. The sequences may be joined directly (i.e., with no intervening amino acids) or may be

sequence is an antigen that contains, within its full length sequence, the recited sequence. The native antigen may, or may not, contain additional amino acid sequence.

An immunogenic portion of a *Leishmania* antigen is a portion that is capable of eliciting an immune response (*i.e.*, cellular and/or humoral) in a presently or previously *Leishmania*-infected patient (such as a human or a dog) and/or in cultures of lymph node cells or peripheral blood mononuclear cells (PBMC) isolated from presently or previously *Leishmania*-infected individuals. The cells in which a response is elicited may comprise a mixture of cell types or may contain isolated component cells (including, but not limited to, T-cells, NK cells, macrophages, monocytes and/or B cells). In particular, immunogenic portions are capable of inducing T-cell proliferation and/or a dominantly Th1-type cytokine response (*e.g.*, IL-2, IFN- γ , and/or TNF- α production by T-cells and/or NK cells; and/or IL-12 production by monocytes, macrophages and/or B cells). Immunogenic portions of the antigens described herein may generally be identified using techniques known to those of ordinary skill in the art, including the representative methods provided herein.

The compositions and methods of the present invention also encompass variants of the above polypeptides. A polypeptide "variant," as used herein, is a polypeptide that differs from the native antigen only in conservative substitutions and/or modifications, such that the ability of the polypeptide to include an immune response is retained. Polypeptide variants preferably exhibit at least about 70%, more preferably at least about 90% and most preferably at least about 95% identity to the identified polypeptides. Alternatively, such variants may be identified by modifying one of the above polypeptide sequences and evaluating the immunogenic properties of the modified polypeptide using, for example, the representative procedures described herein.

A "conservative substitution" is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydrophobic nature of the polypeptide to be substantially unchanged. In general, the following groups of amino acids represent conservative changes: (1) ala, pro, gly, glu, asp, gln,

Figure 29 illustrates the effectiveness of immunization with MAPS-1A plus adjuvant in conferring protection against infection (as measured by footpad swelling) in a murine leishmaniasis model system, as compared to the administration of adjuvant alone.

Figures 30A and B illustrate the proliferation of murine lymph node cultures stimulated with either LcgSP8, LcgSP10 or LcgSP3.

DETAILED DESCRIPTION OF THE INVENTION

As noted above, the present invention is generally directed to compositions and methods for preventing, treating and detecting leishmaniasis, as well as for stimulating immune responses in patients. The compositions of the subject invention include polypeptides that comprise at least an immunogenic portion of a *Leishmania* antigen, or a variant of such an antigen that differs only in conservative substitutions and/or modifications. In one preferred embodiment, compositions of the present invention include multiple polypeptides selected so as to provide enhanced protection against a variety of *Leishmania* species.

Polypeptides within the scope of the present invention include, but are not limited to, polypeptides comprising immunogenic portions of *Leishmania* antigens comprising the sequences recited in SEQ ID NO:2 (referred to herein as M15), SEQ ID NO:4 (referred to herein as Ldp23), SEQ ID NO:6 (referred to herein as Lbhsp83), SEQ ID NO:8 (referred to herein as Lt-210), SEQ ID NO:10 (referred to herein as LbelF4A), SEQ ID NO: 20 (referred to herein as Lmsp1a), SEQ ID NO: 22 (referred to herein as Lmsp9a), SEQ ID NOs: 24 and 26 (referred to herein as MAPS-1A), and SEQ ID NO: 36-42, 49-53 and 55. As used herein, the term "polypeptide" encompasses amino acid chains of any length, including full length proteins (*i.e.*, antigens), wherein the amino acid residues are linked by covalent bonds. Thus, a polypeptide comprising an immunogenic portion of one of the above antigens may consist entirely of the immunogenic portion, or may contain additional sequences. The additional sequences may be derived from the native *Leishmania* antigen or may be heterologous, and such sequences may (but need not) be immunogenic. An antigen "having" a particular

Figure 19 presents a comparison of a Lbhsp83 sequence (SEQ ID NO:6) with homologous sequences from *L. amazonensis* (Lahsp83) (SEQ ID NO:16), *T. cruzi* (Tchsp83) (SEQ ID NO:17) and humans (Huhsp89) (SEQ ID NO:18).

Figure 20 illustrates the reactivity of rabbit sera raised against soluble *Leishmania* antigens with *Leishmania* promastigote lysate (lane 1) and soluble *Leishmania* antigens (lane 2).

Figure 21 shows the cDNA and predicted amino acid sequence for the *Leishmania* antigen Lmspl1a.

Figure 22 shows a Southern blot of genomic DNA from *L. major* digested with a panel of restriction enzymes (lanes 1 to 7) and six other *Leishmania* species digested with PstI (lanes 8 to 13) probed with the full-length cDNA insert of Lmspl1a.

Figure 23 shows a Southern blot of genomic DNA from *L. major* digested with a panel of restriction enzymes, six other *Leishmania* species digested with PstI and the infectious pathogens *T. cruzi* and *T. brucei*, probed with the full-length cDNA insert of the *Leishmania* antigen MAPS-1A.

Figure 24 illustrates the proliferation of PBMC isolated from uninfected individuals, patients with active mucosal leishmaniasis and patients post kala-azar infection, stimulated by MAPS-1A.

Figure 25 illustrates the proliferation of murine lymph node cultures stimulated by MAPS-1A.

Figure 26 illustrates the reactivity of MAPS-1A with sera from human leishmaniasis patients.

Figure 27 illustrates the reactivity of MAPS-1A with sera from mice immunized against and/or infected with leishmaniasis.

Figure 28 illustrates the effectiveness of immunization with either soluble *Leishmania* antigens or a mixture of Ldp23, LbeiF4A and M15 plus adjuvant in conferring protection against infection (as measured by footpad swelling) in a murine leishmaniasis model system, as compared to the administration of adjuvant alone.

infected and control individuals in response to stimulation with parasite lysate or the indicated polypeptides.

Figure 11 illustrates the levels of IL-10 p40 (in pg/mL) in the supernatant of PBMC cultures from *L. braziliensis*-infected individuals and uninfected controls 72 hours following stimulation with parasite promastigote lysate (Lb), Lbhsp83a or Lbhsp83b.

Figure 12 presents the reactivities of sera from *L. braziliensis* infected-patients with representative polypeptides of the present invention in a standard ELISA. Values are expressed as absorbance at 405 nm.

Figures 13A and 13B illustrate the level of secreted IL-4 and IFN- γ (in pg/mL) stimulated in mouse lymph node cultures by the addition of representative polypeptides of the present invention.

Figure 14 shows the level of IFN- γ (in pg/mL) secreted by *Leishmania*-infected and uninfected human PBMC stimulated by the *Leishmania* antigen M15, as compared to the levels stimulated by *L. major* lysate and L-Rack, an antigen that does not appear to be recognized by *Leishmania*-infected humans.

Figure 15 shows the level of IFN- γ (in pg/mL) secreted by infected and uninfected human PBMC stimulated by soluble *Leishmania* antigens (S antigens), as compared to the levels stimulated by *L. major* lysate and L-Rack.

Figure 16 illustrates the proliferation of murine lymph node cultures stimulated by the addition of representative polypeptides of the present invention. Values are expressed as cpm.

Figure 17 shows the proliferation of human PBMC, prepared from *Leishmania*-immune and uninfected individuals, stimulated by M15 as compared to the proliferation stimulated by *L. major* lysate and L-Rack. Values are expressed as cpm.

Figure 18 illustrates the proliferation of human PBMC, prepared from *Leishmania*-infected and uninfected individuals, stimulated by soluble *Leishmania* antigens as compared to the proliferation stimulated by culture medium, *L. major* lysate and L-Rack. Values are expressed as cpm.

and 3 refer to RNA obtained from promastigotes at the logarithmic growth phase, promastigotes at the stationary growth phase and amastigote forms, respectively.

Figure 5 shows a Western blot analysis of *L. donovani* promastigote antigens incubated with pre-immune rabbit serum (lane A) or with anti-Ldp23 rabbit antiserum (lane B).

Figure 6 illustrates the surface expression of Ldp23 on live *L. donovani* promastigotes. The dotted line shows the indirect immunofluorescence performed using pre-immune mouse serum and the solid line shows the result obtained with mouse anti-GST-Ldp23 antiserum. Fluorescence intensity was analyzed by FACScan.

Figure 7 shows the stimulation of *Leishmania*-specific T-cell proliferation by Ldp23. The results are presented as relative cell number as a function of fluorescence intensity. T-cells (10^5 /well) were purified from lymph nodes of BALB/c mice immunized in the foot pad with *L. donovani* promastigotes in CFA and were cultured with various concentrations of the purified recombinant Ldp23 in the presence of 2×10^5 Mitomycin C-treated normal BALB/c spleen mononuclear cells. Proliferation of T-cells was measured at 27 hours of culture. Values are expressed as cpm and represent the mean of [3 H]TdR incorporation of triplicate cultures.

Figure 8 illustrates Ldp23-induced cytokine production by lymph node cells of BALB/c mice. Cultures were incubated with varying amounts of Ldp23 or *Leishmania* lysate, presented as $\mu\text{g/mL}$, and were assayed by ELISA for the production of interferon- γ (panel A) or interleukin-4 (panel B), both of which are shown as ng/mL .

Figure 9 shows the PCR amplification of cytokine mRNAs isolated from mucosal leishmaniasis (Panel A) and cutaneous leishmaniasis (panel B) patient PBMC before and after stimulation with representative polypeptides of the present invention. Lanes O and - indicate the level of PCR products at the initiation of culture and after 72 hours of culture, respectively, in the absence of added polypeptide; lanes Lb, 83a and 83b indicate the level of PCR products following culturing of PBMC with *L. braziliensis* lysate, and the *Leishmania* antigens Lbhsp83a and Lbhsp83b, respectively.

Figure 10 presents a comparison of the levels of interferon- γ (panel A) and TNF- α (panel B) in the supernatants of 72 hour PBMC cultures from *Leishmania*-

above; and (b) an apparatus sufficient to contact the pharmaceutical composition with the dermal cells of a patient.

In further aspects, the present invention provides methods for stimulating a cellular and/or humoral immune response in a patient, comprising administering to a patient a pharmaceutical composition or vaccine as described above.

In a related aspect, methods are provided for treating a patient afflicted with a disease responsive to IL-12 stimulation, comprising administering to a patient a pharmaceutical composition or vaccine as described above.

These and other aspects of the present invention will become apparent upon reference to the following detailed description and attached drawings. All references disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the stimulation of proliferation of T-cells obtained from *L. donovani*-immunized BALB/c mice (represented by stimulation index) by *L. donovani*-infected macrophages after incubation for 24, 48 and 72 hours.

Figure 2 illustrates representative HPLC profiles of peptides isolated from MHC class II molecules of P388D1 macrophages. Panel A shows peptides isolated from uninfected macrophages and panel B shows peptides isolated from *L. donovani* infected macrophages. The arrows in panel B indicate peptide peaks present only in the infected macrophage preparation.

Figure 3 illustrates the expression and purification of the *Leishmania* antigen Ldp23 as a recombinant fusion protein. Panel A shows a Coomassie blue-stained SDS-PAGE gel of lysed *E. coli* without (lane 1) and with (lane 2) IPTG induction of Ldp23 expression. Arrow indicates the recombinant fusion protein. Panel B shows the fusion protein following excision from a preparative SDS-PAGE gel, electroelution, dialysis against PBS and analytical SDS-PAGE.

Figure 4 presents a Northern blot analysis of total RNA prepared from *L. donovani*, *L. major*, *L. amazonensis* and *L. pifanoi* with a ³²P labeled Ldp23 gene. 1, 2

sequence selected from the group consisting of SEQ ID Nos: 2, 4, 20, 22, 24, 26, 36-38, 41, 50-53 and 82. DNA sequences encoding the above polypeptides, recombinant expression vectors comprising these DNA sequences and host cells transformed or transfected with such expression vectors are also provided.

5 In related aspects, the present invention provides pharmaceutical compositions which comprise one or more of the polypeptides described herein, or a DNA molecule encoding such polypeptides, and a physiologically acceptable carrier. Vaccines which comprise one or more such polypeptides or DNA molecules, together with a non-specific immune response enhancer are also provided. In specific
10 embodiments of these aspects, the *Leishmania* antigen has an amino acid sequence selected from the group consisting of SEQ ID Nos: 2, 4, 20, 22, 24, 26, 36-38, 41, 50-53 and 82.

In still further related embodiments, the pharmaceutical compositions and vaccines comprise at least two different polypeptides, each polypeptide comprising
15 an immunogenic portion of a *Leishmania* antigen having an amino acid sequence selected from the group consisting of sequences recited in SEQ ID Nos: 2, 4, 6, 8, 10, 20, 22, 24, 26, 36-38, 41, 50-53, 82, and variants thereof that differ only in conservative substitutions and/or modifications. In other embodiments, the inventive pharmaceutical compositions comprise one or more of the inventive polypeptides in combination with a
20 known *Leishmania* antigen.

In yet other related embodiments, the pharmaceutical compositions and vaccines comprise soluble *Leishmania* antigens.

In another aspect, the present invention provides methods for inducing protective immunity against leishmaniasis in a patient, comprising administering to a
25 patient a pharmaceutical composition or vaccine as described above.

In further aspects, methods and diagnostic kits are provided for detecting *Leishmania* infection in a patient. The methods comprise: (a) contacting dermal cells of a patient with a pharmaceutical composition as described above; and (b) detecting an immune response on the patient's skin, therefrom detecting *Leishmania* infection in the
30 patient. The diagnostic kits comprise: (a) a pharmaceutical composition as described

Leishmaniasis is a serious problem in much of the world, including Brazil, China, East Africa, India and areas of the Middle East. The disease is also endemic in the Mediterranean region, including southern France, Italy, Greece, Spain, Portugal and North Africa. The number of cases of leishmaniasis has increased dramatically in the last 20 years, and millions of cases of this disease now exist worldwide. About 2 million new cases are diagnosed each year, 25% of which are visceral leishmaniasis. There are, however, no vaccines or effective treatments currently available.

Accurate diagnosis of leishmaniasis is frequently difficult to achieve.

10 There are 20 species of *Leishmania* that infect humans, including *L. donovani*, *L. chagasi*, *L. infantum*, *L. major*, *L. amazonensis*, *L. braziliensis*, *L. panamensis*, *L. mexicana*, *L. tropica*, and *L. guyanensis*, and there are no distinctive signs or symptoms that unambiguously indicate the presence of *Leishmania* infection. Parasite detection methods have been used, but such methods are neither sensitive nor clinically practical.

15 Current skin tests typically use whole or lysed parasites. Such tests are generally insensitive, irreproducible and prone to cross-reaction with a variety of other diseases. In addition, the preparations employed in such tests are often unstable. Thus, there is a need for improved methods for the detection of *Leishmania* infection.

Current experimental vaccines consisting of whole organisms have not proven effective in humans. Accordingly, there remains a need in the art for vaccines to prevent leishmaniasis in humans and dogs, and for improved therapeutic compositions for the treatment of leishmaniasis.

SUMMARY OF THE INVENTION

25 Briefly stated, the present invention provides compositions and methods for preventing, treating and detecting leishmaniasis, as well as for stimulating immune responses in patients. In one aspect, polypeptides are provided which comprise at least an immunogenic portion of a *Leishmania* antigen, or a variant of such an antigen that differs only in conservative substitutions and/or modifications. In specific

30 embodiments of the invention, the *Leishmania* antigen comprises an amino acid

LEISHMANIA ANTIGENS FOR USE IN THE THERAPY AND
DIAGNOSIS OF LEISHMANIASIS

REFERENCE TO RELATED APPLICATIONS

5

TECHNICAL FIELD

The present invention relates generally to compositions and methods for preventing, treating and detecting leishmaniasis, and for stimulating immune responses in patients. The invention is more particularly related to polypeptides comprising an immunogenic portion of a *Leishmania* antigen or a variant thereof, and to vaccines and pharmaceutical compositions comprising one or more such polypeptides. The vaccines and pharmaceutical compositions may be used, for example, for the prevention and therapy of leishmaniasis, as well as for the detection of *Leishmania* infection.

15

BACKGROUND OF THE INVENTION

Leishmania organisms are intracellular protozoan parasites of macrophages that cause a wide range of clinical diseases in humans and domestic animals, primarily dogs. In some infections, the parasite may lie dormant for many years. In other cases, the host may develop one of a variety of forms of leishmaniasis. For example, the disease may be asymptomatic or may be manifested as subclinical visceral leishmaniasis, which is characterized by mild symptoms of malaise, diarrhea and intermittent hepatomegaly. Patients with subclinical or asymptomatic disease usually have low antibody titers, making the disease difficult to detect with standard techniques. Alternatively, leishmaniasis may be manifested as a cutaneous disease, which is a severe medical problem but is generally self-limiting, or as a highly destructive mucosal disease, which is not self-limiting. Finally, and most seriously, the disease may be manifested as an acute visceral infection involving the spleen, liver and lymph nodes, which, untreated, is generally a fatal disease. Symptoms of acute visceral leishmaniasis include hepatosplenomegaly, fever, leukopenia, anemia and hypergammaglobulinemia.

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5/10, A61K 39/008, A61K 48/00

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(74) Agents: MAKI, David, J. et al.; Seed and Berry LLP, 6300 Columbia Center, 701 Fifth Avenue, Seattle, WA 98104-7092 (US).

(81) Designated States: BR, MX, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published

With international search report.

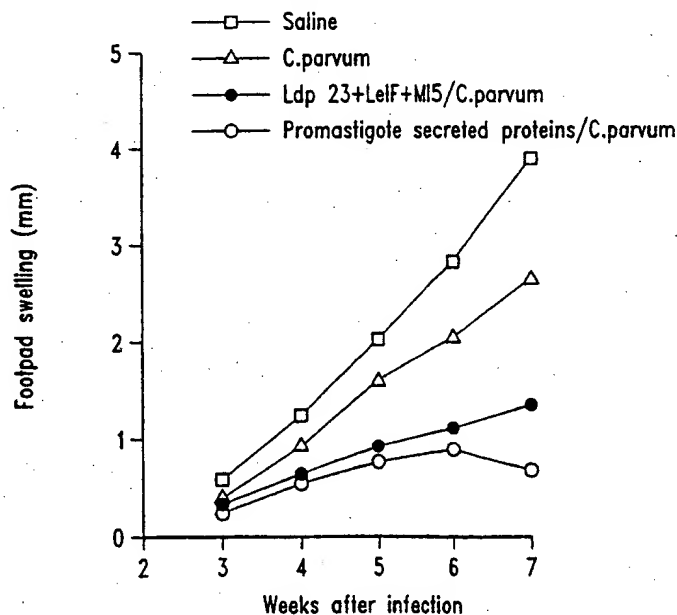
(88) Date of publication of the international search report:

29 October 1998 (29.10.98)

(54) Title: LEISHMANIA ANTIGENS FOR USE IN THE THERAPY AND DIAGNOSIS OF LEISHMANIASIS

(57) Abstract

Compositions and methods for preventing, treating and detecting leishmaniasis and stimulating immune responses in patients are disclosed. The compounds provided include polypeptides that contain at least an immunogenic portion of one or more *Leishmania* antigens, or a variant thereof. Vaccines and pharmaceutical compositions comprising such polypeptides, or DNA molecules encoding such polypeptides, are also provided and may be used, for example, for the prevention and therapy of leishmaniasis, as well as for the detection of *Leishmania* infection.

Protection against infection with *L. major* in BALB/c mice immunized leishmanial antigens.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internal Application No

PCT/US 98/03002

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9529239 A	02-11-1995	AU 2427695 A	16-11-1995
		BR 9507505 A	02-09-1997
		CA 2188543 A	02-11-1995
		CN 1149887 A	14-05-1997
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		NO 964468 A	20-12-1996

WO 9711180 A	27-03-1997	AU 7242796 A	09-04-1997
		EP 0854924 A	29-07-1998

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Idem as subject 18, for SEQ ID NO:85

28. Claims: Partially 56-62

Idem as subject 18, for SEQ ID NO:86

29. Claims: Partially 56-62

Idem as subject 18, for SEQ ID NO:87

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Leishmania antigen comprising SEQ ID NO:20, variant thereof,
DNA molecule encoding it, expression vector and host cell
comprising the same, pharmaceutical compositions and
vaccines comprising the polypeptide, vaccine comprising the
DNA molecule, diagnostic kit

18. Claims: Partially 56-62

Pharmaceutical compositions and vaccines comprising a
polypeptide comprising an immunogenic portion of a
Leishmania antigen comprising SEQ ID NO:39 or a variant
thereof

19. Claims: Partially 56-62

Idem as subject 18, for SEQ ID NO:42

20. Claims: Partially 56-62

Idem as subject 18, for SEQ ID NO:55

21. Claims: Partially 56-62

Idem as subject 18, for SEQ ID NO:61

22. Claims: Partially 56-62

Idem as subject 18, for SEQ ID NO:62

23. Claims: Partially 56-62

Idem as subject 18, for SEQ ID NO:80

24. Claims: Partially 56-62

Idem as subject 18, for SEQ ID NO:81

25. Claims: Partially 56-62

Idem as subject 18, for SEQ ID NO:83

26. Claims: Partially 56-62

Idem as subject 18, for SEQ ID NO:84

27. Claims: Partially 56-62

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Idem as subject 3, for SEQ ID NO:38

9. Claims: Partially 13, 16-19, 25, 27-31, 33-40, 42-55

Idem as subject 3, for SEQ ID NO:41

10. Claims: Partially 13, 16-19, 25, 27-31, 33-40, 42-55

Idem as subject 3, for SEQ ID NO:49

11. Claims: Partially 13, 16-19, 25, 27-31, 33-40, 42-55

Idem as subject 3, for SEQ ID NO:50

12. Claims: Partially 13, 16-19, 25, 27-31, 33-40, 42-55

Idem as subject 3, for SEQ ID NO:51

13. Claims: Partially 13, 16-19, 25, 27-31, 33-40, 42-55

Idem as subject 3, for SEQ ID NO:52

14. Claims: Partially 13, 16-19, 25, 27-31, 33-40, 42-55

Idem as subject 3, for SEQ ID NO:53

15. Claims: Partially 13, 16-19, 25, 27-29, 31, 33, 35-40,
42-55

Idem as subject 3, for SEQ ID NO:82

16. Claims: 14, 15, and partially 16, 25, 27-29, 31, 33, 35-40,
42-55

Antigenic epitope of Leishmania antigen comprising SEQ ID NO:43, polypeptide comprising at least two such contiguous epitopes, DNA molecule encoding it, pharmaceutical compositions and vaccines comprising the polypeptide, vaccine comprising the DNA molecule, diagnostic kit

17. Claims: 20-24, 26, 32, 41 and partially 27-30, 33-39, 42-55

Polypeptide comprising an immunogenic portion of a

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-6, and partially 25, 27-29, 31, 33, 35-40, 42-55

Polypeptide comprising an immunogenic portion of a Leishmania antigen having SEQ ID NO:2, variant thereof, DNA molecule encoding it, expression vector and host cell comprising the same, pharmaceutical compositions and vaccines comprising the polypeptide, vaccine comprising the DNA molecule, diagnostic kit

2. Claims: 7-12, and partially 25, 27-29, 31, 33, 35-40, 42-55

Polypeptide comprising an immunogenic portion of a Leishmania antigen having SEQ ID NO:4, variant thereof, DNA molecule encoding it, expression vector and host cell comprising the same, pharmaceutical compositions and vaccines comprising the polypeptide, vaccine comprising the DNA molecule, diagnostic kit

3. Claims: Partially 13, 16-19, 25, 27-31, 33-40, 42-55

Polypeptide comprising an immunogenic portion of a Leishmania antigen comprising SEQ ID NO:22, variant thereof, DNA molecule encoding it, expression vector and host cell comprising the same, pharmaceutical compositions and vaccines comprising the polypeptide, vaccine comprising the DNA molecule, diagnostic kit

4. Claims: Partially 13, 16-19, 25, 27-31, 33-40, 42-55

Idem as subject 3, for SEQ ID NO:24

5. Claims: Partially 13, 16-19, 25, 27-31, 33-40, 42-55

Idem as subject 3, for SEQ ID NO:26

6. Claims: Partially 13, 16-19, 25, 27-31, 33-40, 42-55

Idem as subject 3, for SEQ ID NO:36

7. Claims: Partially 13, 16-19, 25, 27-31, 33-40, 42-55

Idem as subject 3, for SEQ ID NO:37

8. Claims: Partially 13, 16-19, 25, 27-31, 33-40, 42-55

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 98/ 03002

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see continuation sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

see continuation sheet, subject 1

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Internat I Application No

PCT/US 98/03002

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	<p>WO 97 11180 A (CORIXA CORPORATION) 27 March 1997</p> <p>see page 2, line 20 - page 3, line 37 see page 6, line 16 - page 7, line 20 see page 9, line 4 - line 19 see page 12, line 20 - page 14, line 17 see page 15, line 3 - page 16, line 26 see page 17, line 8 - line 30; claims; examples 1,7,8</p> <p>-----</p>	<p>1-6,25, 27-29, 31, 35-40, 42-55</p>

INTERNATIONAL SEARCH REPORT

Internati Application No
PCT/US 98/03002

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/30 C07K14/44 C12N15/63 C12N5/10 A61K39/008
A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 95 29239 A (CORIXA CORPORATION) 2 November 1995 see page 2, line 17 - page 3, line 37 see page 5, line 8 - page 23, line 9; examples --- -/--	1-6,25, 27-29, 31, 35-40, 42-55

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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E earlier document but published on or after the international filing date

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O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

& document member of the same patent family

Date of the actual completion of the international search

28 May 1998

Date of mailing of the international search report

27. 08. 1998

Name and mailing address of the ISA

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Authorized officer

MONTERO LOPEZ B.

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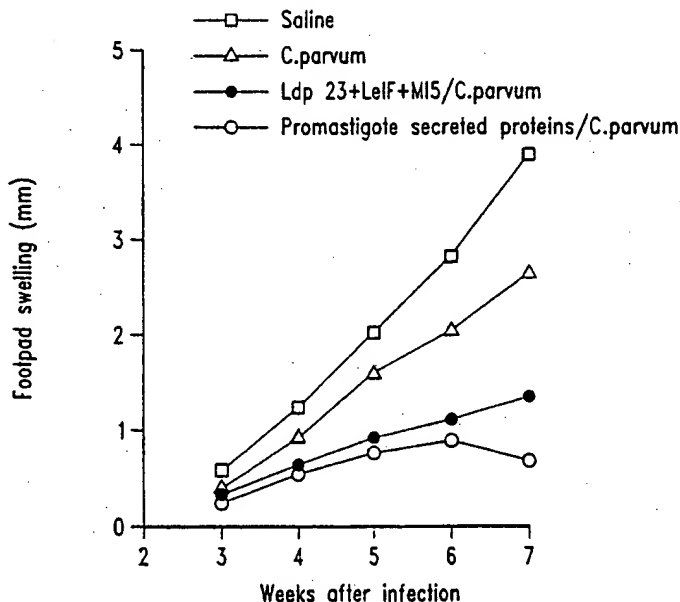
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(51) International Patent Classification ⁶ : C12N 15/30, C07K 14/44, C12N 15/63 5/10, A61K 39/008, A61K 48/00		A3	(11) International Publication Number: WO 98/35045
(21) International Application Number: PCT/US98/03002		(43) International Publication Date: 13 August 1998 (13.08.98)	
(22) International Filing Date: 12 February 1998 (12.02.98)		(81) Designated States: BR, MX, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).	
(30) Priority Data: 08/798,841 12 February 1997 (12.02.97) US 08/920,609 27 August 1997 (27.08.97) US		Published With international search report.	
(71) Applicant: CORIXA CORPORATION [US/US]; Suite 200, 1124 Columbia Street, Seattle, WA 98104 (US).		(88) Date of publication of the international search report: 29 October 1998 (29.10.98)	
(72) Inventors: REED, Steven, G.; 2843 122nd Place N.E., Bellevue, WA 98005 (US). CAMPOS-NETO, Antonio; 9308 Midship Court N.E., Bainbridge Island, WA 98110 (US). WEBB, John, R.; 15314 Silverfirs Drive, Everett, WA 98208 (US). DILLON, Davin, C.; 21607 N.E. 24th Street, Redmond, WA 98053 (US). SKEIKY, Yasir, A.; 8327 - 25th Avenue N.W., Seattle, WA 98107 (US).			
(74) Agents: MAKI, David, J. et al.; Seed and Berry LLP, 6300 Columbia Center, 701 Fifth Avenue, Seattle, WA 98104-7092 (US).			

(54) Title: LEISHMANIA ANTIGENS FOR USE IN THE THERAPY AND DIAGNOSIS OF LEISHMANIASIS

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Protection against infection with *L. major* in BALB/c mice immunized leishmanial antigens.

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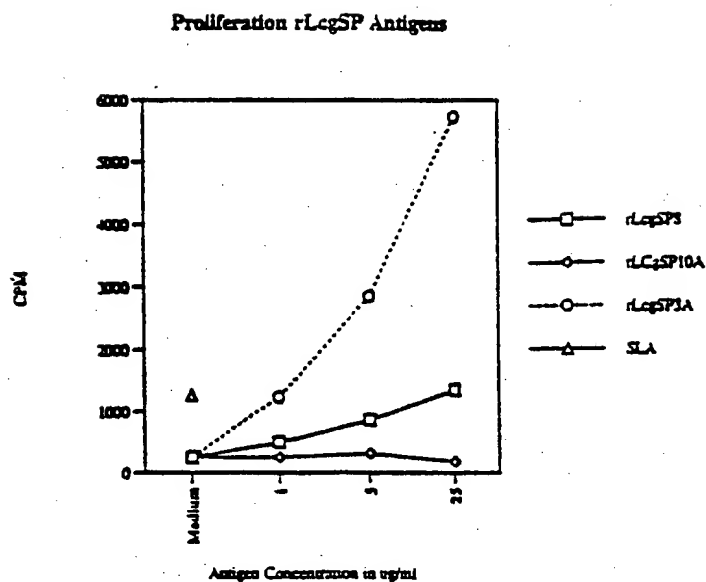


FIGURE 30A

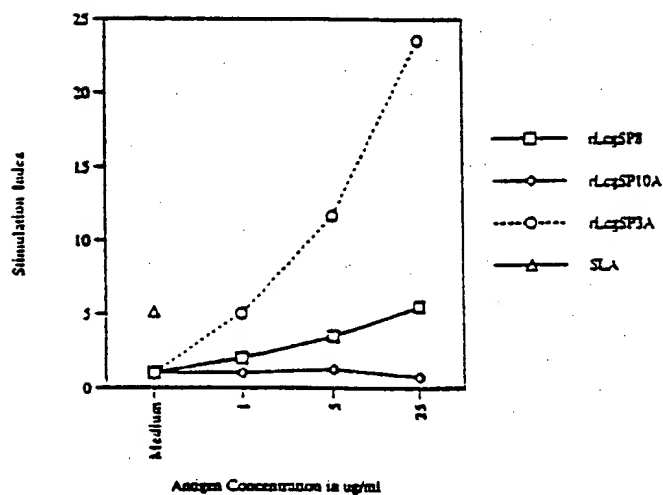


Figure 30B

FIG. 30 A and B

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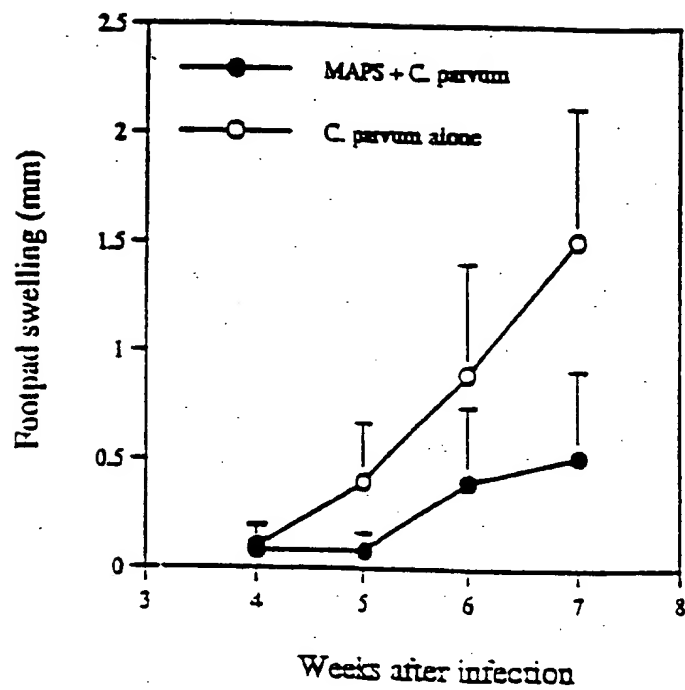


FIG. 29

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FIG. 28

Protection against infection with *L. major* in BALB/c mice immunized leishmanial antigens.

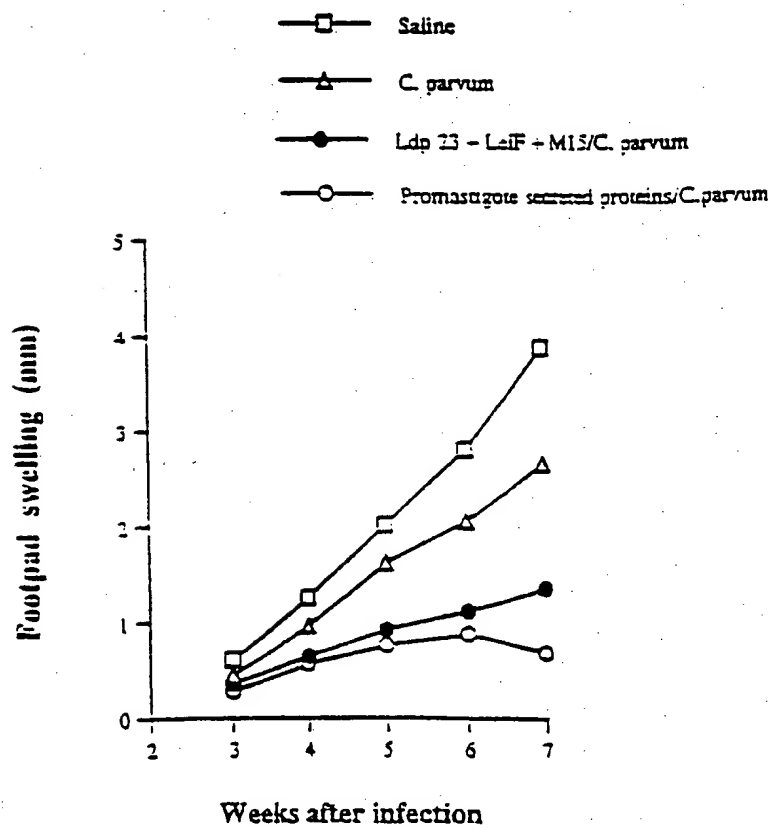


Figure 28

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Antibody Response of Immunized/li BALB/c Mice to MAPS

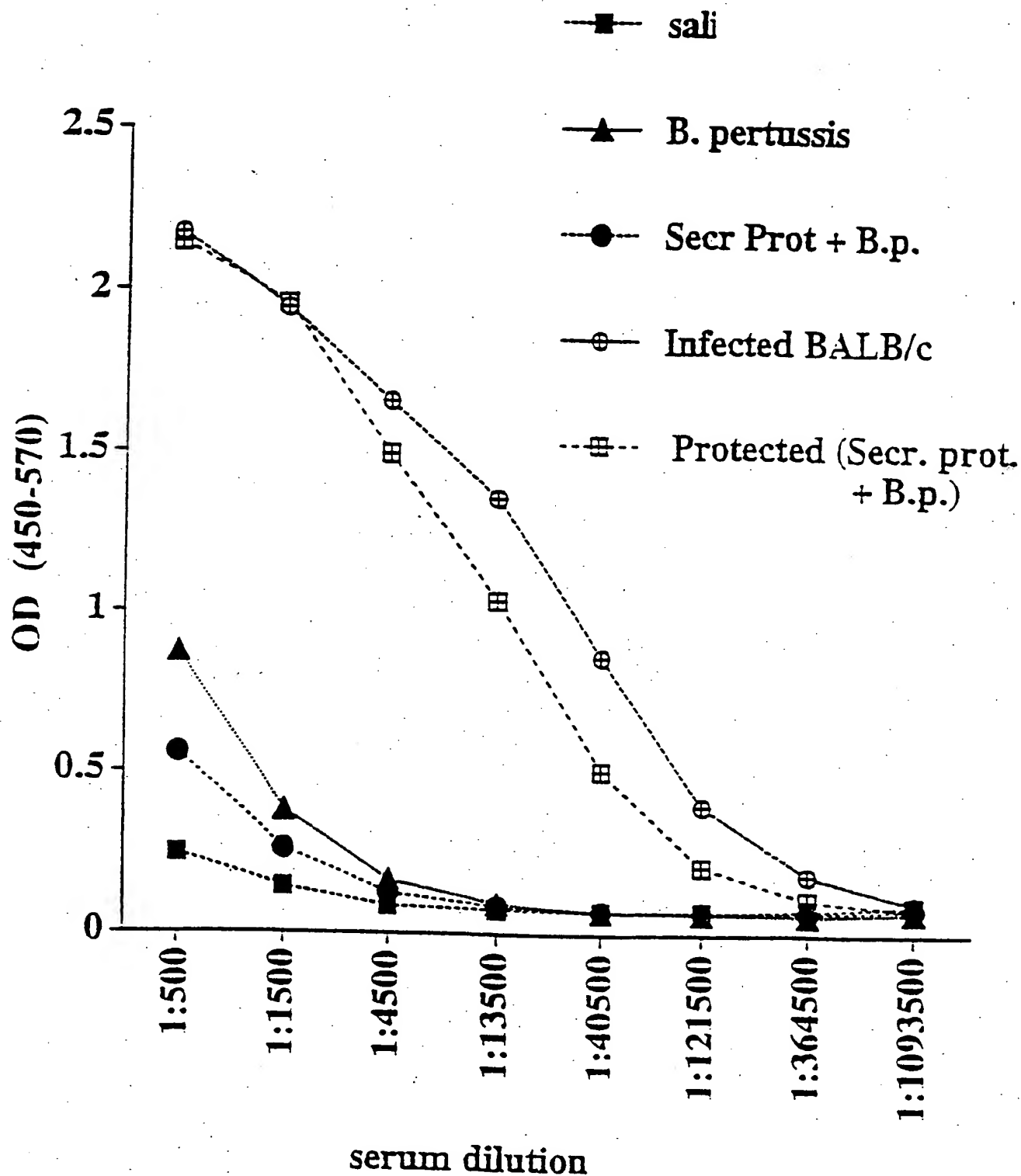


FIG. 27

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Proliferative Responses of *L. major*-infected BALE
(20 days post-infection) to recombinant MAPS protein

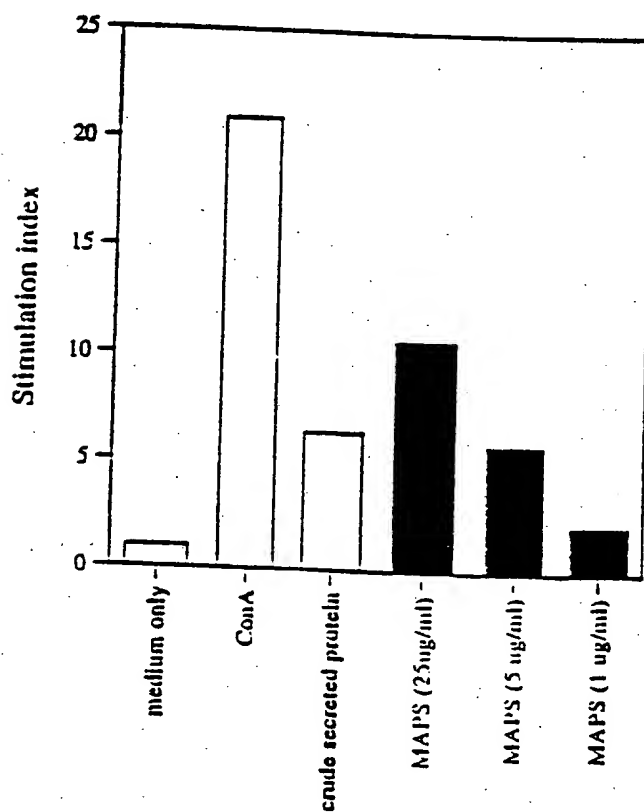
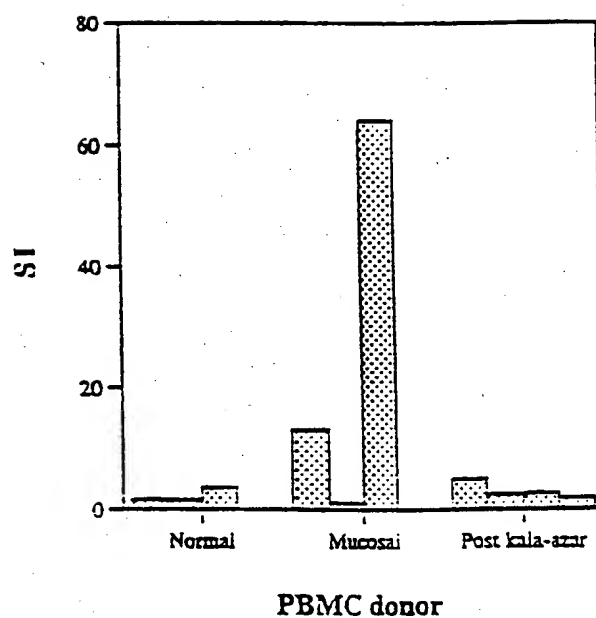


FIG. 25

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FIG. 24

Proliferative response of human PBMC to MAPS

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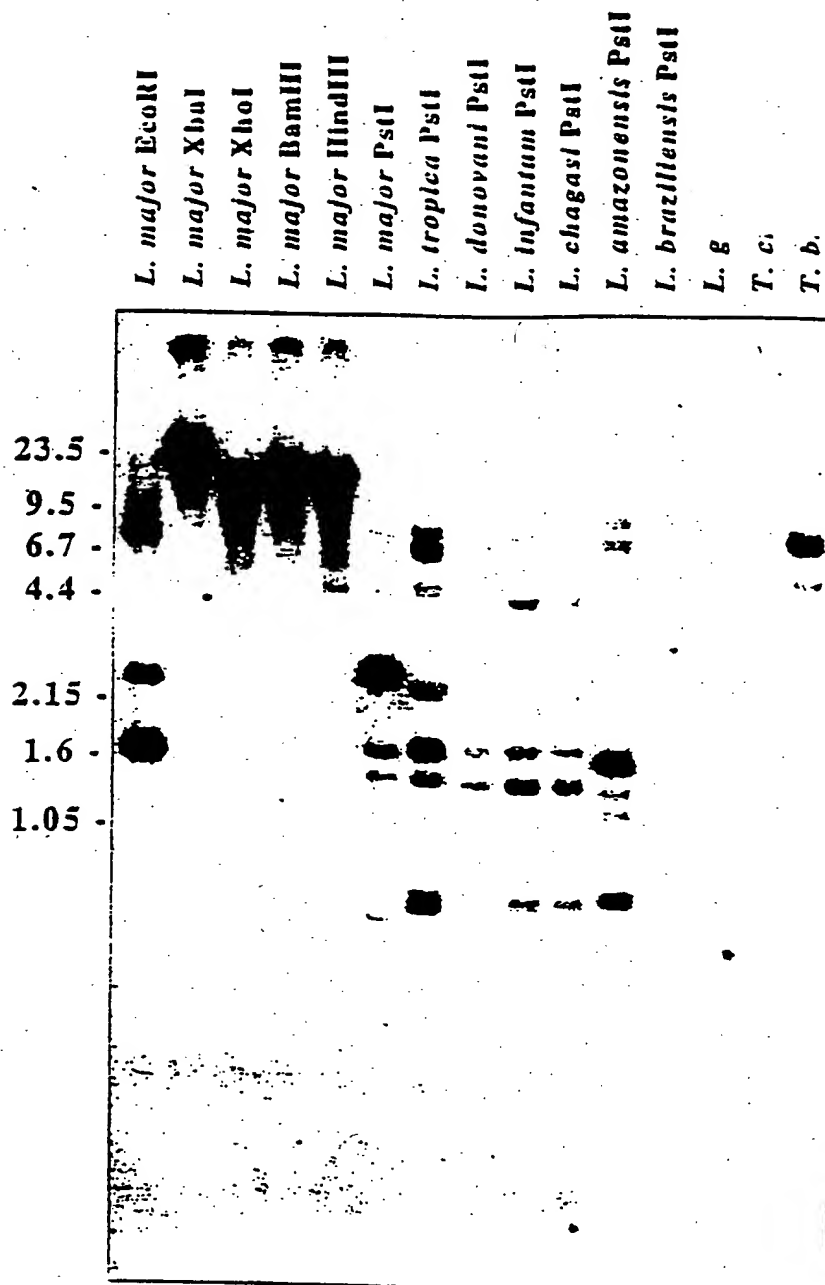


FIG. 23

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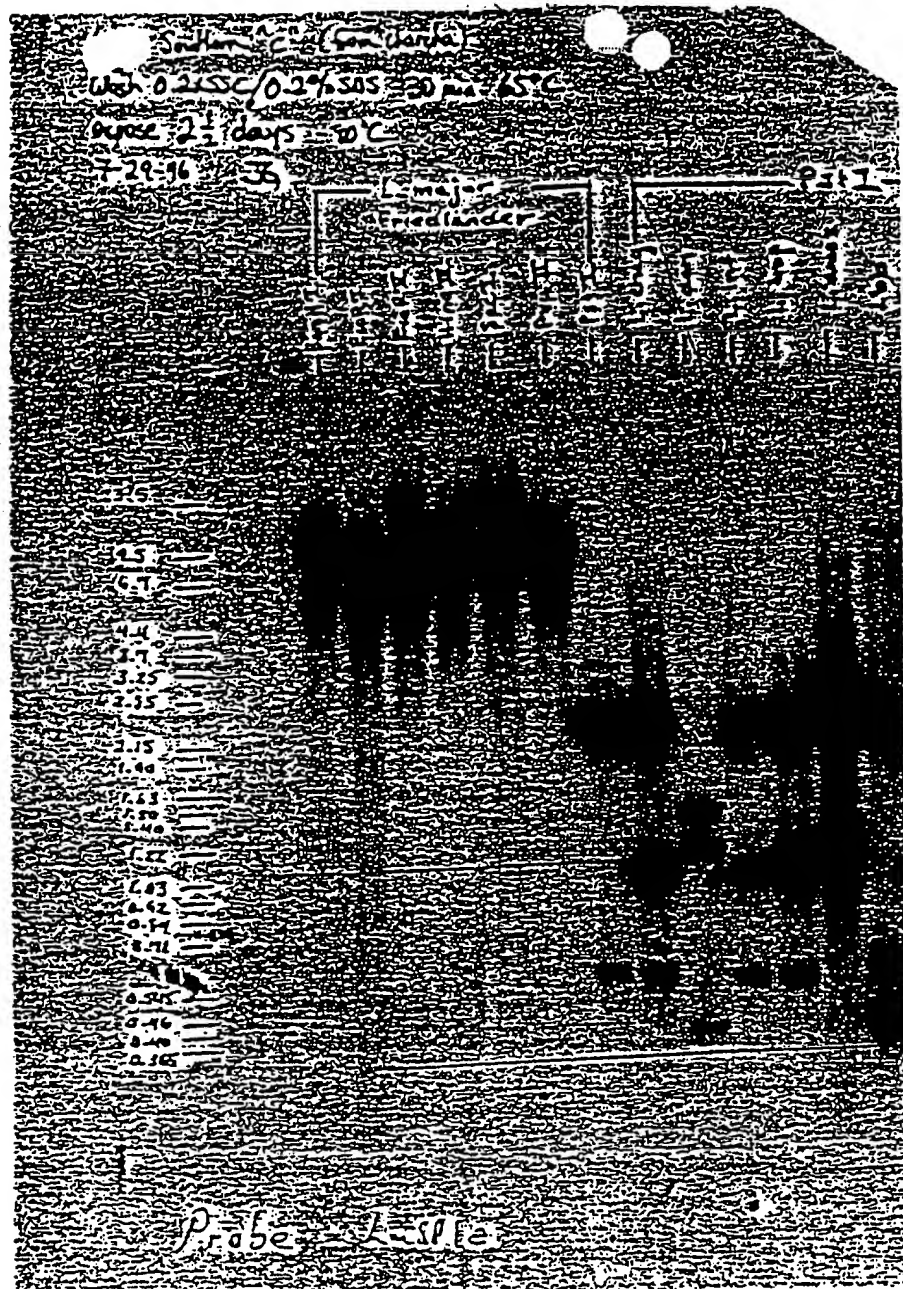


FIG. 22

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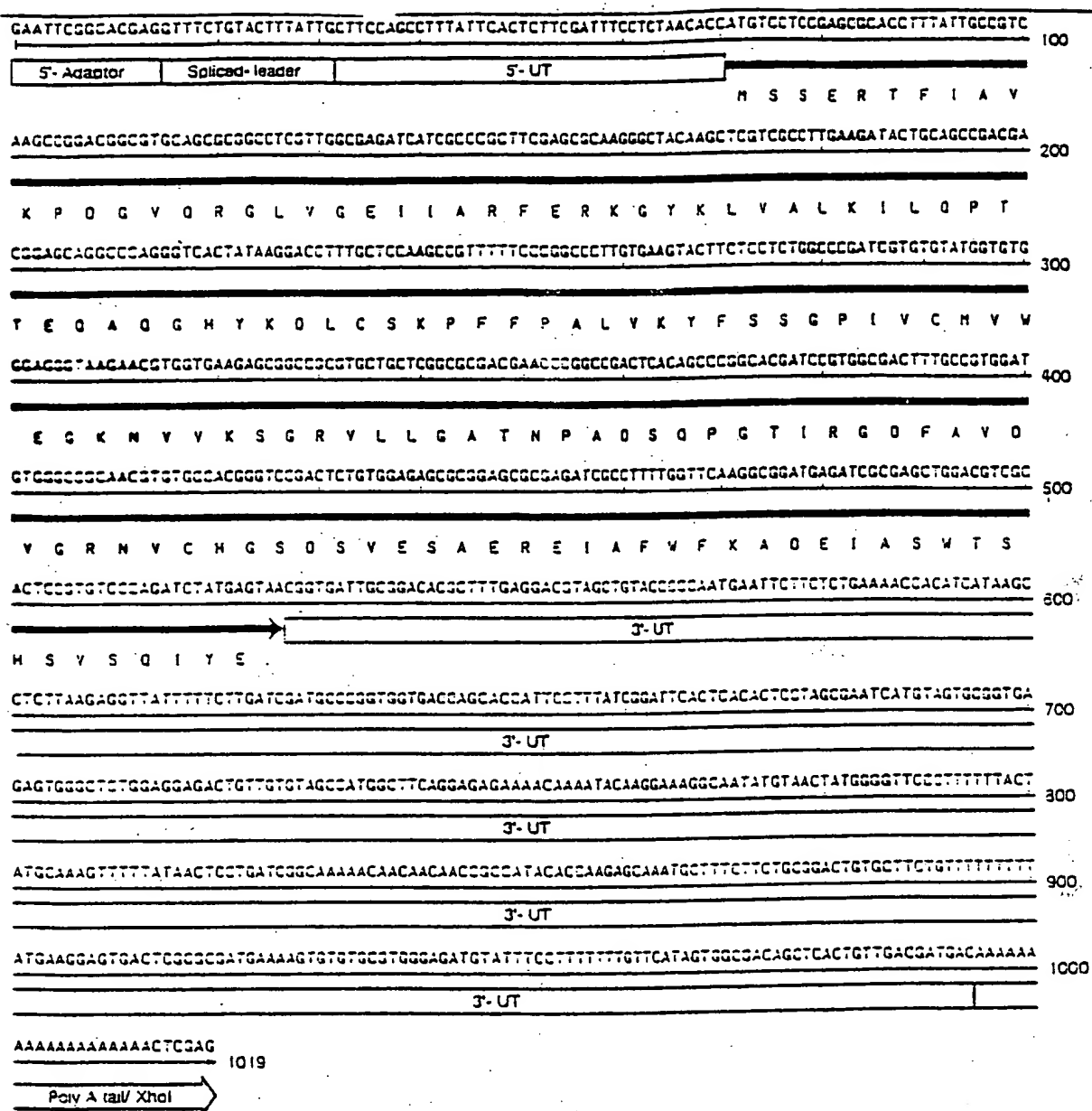
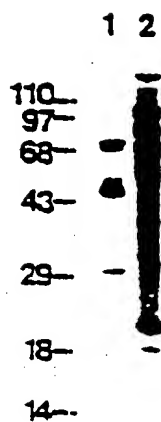


FIG. 21

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**FIG. 20**

HTETFAQAEINQLHSLIINTFYSHKEIFLNUVISHASDACUKIRYQ.....NA.R.C.....E.....05
05
 HPEETOTQQRHBEEN.....A.....EL.....S.....I.....SK.USUKE.HHNL.II.QUNA.IV.T.....85
100

ADLVHHLUTIARSOTKAFHEALGDDHSHIGQFUVGFYSAYLVANHTVNVSKHSHENY-HESSAGUTFTITSVQESDHKHTSTTLII.KEDQVEYLEE 184
A.....T.....V.V.....AP.....LPAN.....A 185
E.....U.....T.....V.PTPDC.L.....RIV.....185
I.....K.....Q.A.I.....EK.IT.H.D.Q.A.....S.VRTDTGP.O.....KVI.....T.....200

RRVKEI:KKHSEFIGYDIELHVEKTAKEVNTDE---DEEENESKKKSCWDEEPKVEENTEDD-ED-KKKTKKKVKEVKT-YEVK---NKIKPLWTRD 274
T.....A.ADE.GE.....E.....T.E---Q---272
AT.....D.....AAATKHEUB.....KHDAE.GE.....TQE-FV.Q---275
I.V.....Q.....P.T.F.....END.....S.DESEK.DKE.E.E.EEKESEDKPEI.DVUSHE..E.DGD..K.KKI.EK.ID.EEL..T..I..H 300

TKDVTKEEYALFYKAIBHDWETAATKIFSVGOQLEFRATIAFVRKRAFFHIFERHKKRHHIKLYNIRVFINDCEDLCPDHLQFVKGVNDSEDLPLHISR 374
 P.....PP.....H.....I.....
 P.....EPH.B.....I.....S.....B.A.R.....372
 PD.I.H...GE...SLT...HL.V.....IL..R.....I..HL..K.....E.I.EY.N.IN.....400

ENLOQNKILKVIRKHIIVKKCLELFEBEIAENKEDYKQFYEUFIKHIKLOIHEDTNIIRKKI.HELL.HFTSTESOEHTTLKUYVTHHKPEQKSIYYITDSKK 474
H..V.....
A.....K.....V.....S.....H.B...D.....ED..C..V.....472
 H..S.....L.....T.L.D.N.K...S.....SQ.....S...Y.TSA..D..V8...C...EN..H.....ET.D 500

KLESPFIEKARCOLEVLHTERIDEYVHQVKDFEUKKFACTKEGVHFESEEEKKQHEEKKAAEKKICKTHKEVLQDKVEKVTVERLI.TSPCILV 574
Q.K.R.F.....Y.....E.T.....S.....572
 T.....Q..R.F.....I.....T.....E.T.Y.H..A..D.....V.....A.....575
 QVAN.A.V.RL.KH...IV.I.....CV..I..E..Q.TIVSV...I.H.P.D.....KQ...TKF.H..I..DI.EK...V..H..V...C...600

TSEFHSAMHEQIHRIHQALADZ6HQAQVHVSCKTHENVHNIHFIKELHNVKADENHKKVKKOLVFLI.FDTSILIT.SUQJLNDPTGYACERINHIIKLOLSIDE 674
H.....H.....I..K.....E.....671
SA..H.....I..A..V..K.....Y..A.....T.....S.....H.....D 675
 TY..T.H..H..KA...H.THO..AA..III.I...S..ET..QKA..K..S.....I..YE.A..S...S.E..QTH.N..Y.....GI...700

EE--EEVA-EAPPAEAPAEVNTAGTSSHEQVD 703 lhhsp81
 ..E--E.V..AV..T.....L.. 701 lhhsp81
 D---HGH2..E..A.V..PV..... 704 Tchlsp81
 NDPTADITSA.VTE.HP.I.GUUD..R..E.. 714 lhhsp89

FIG. 19

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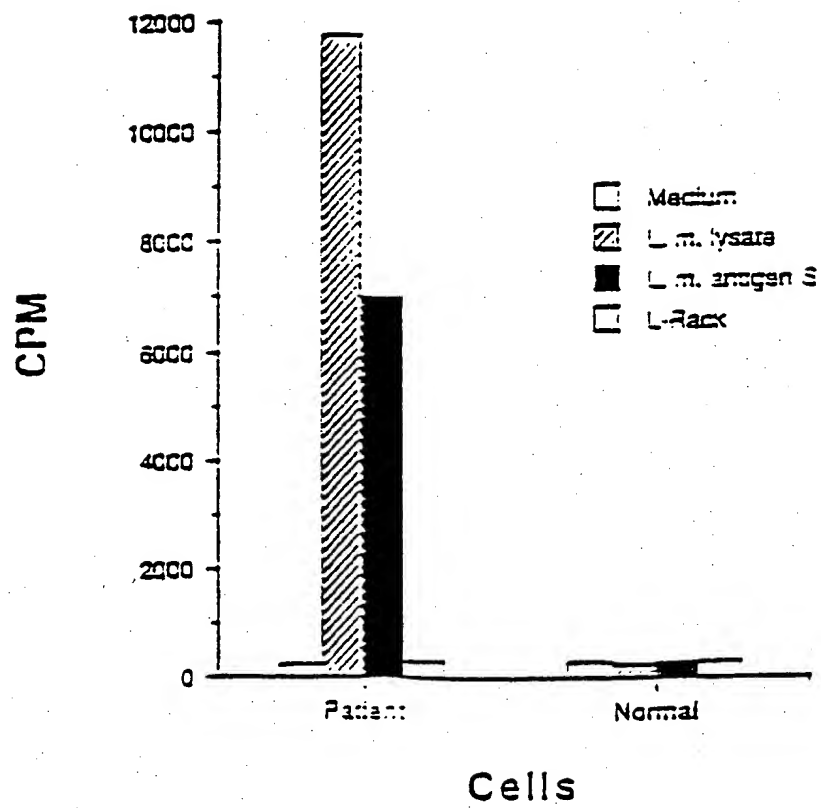


FIG. 18

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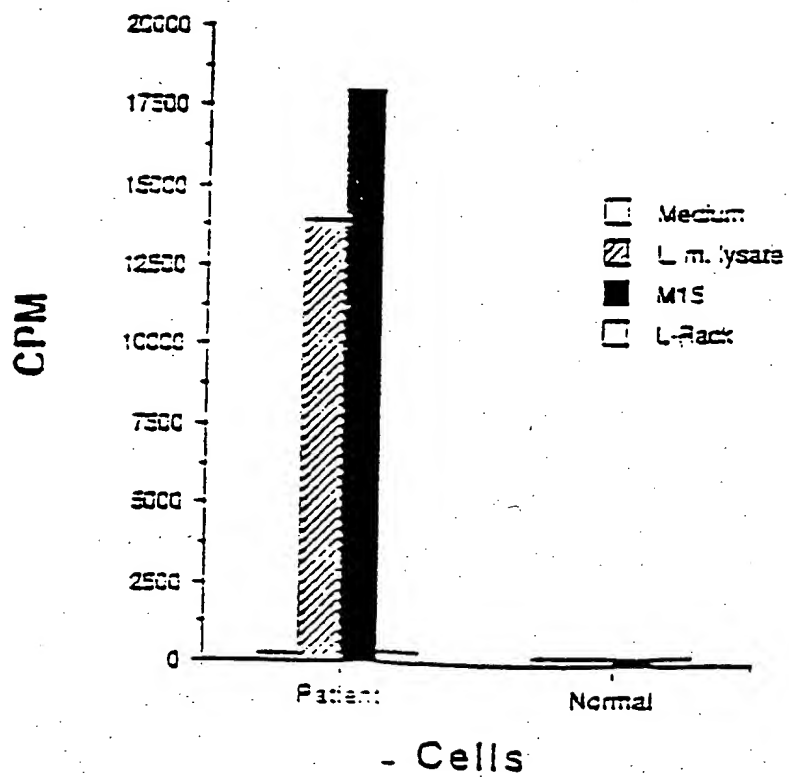


FIG. 17

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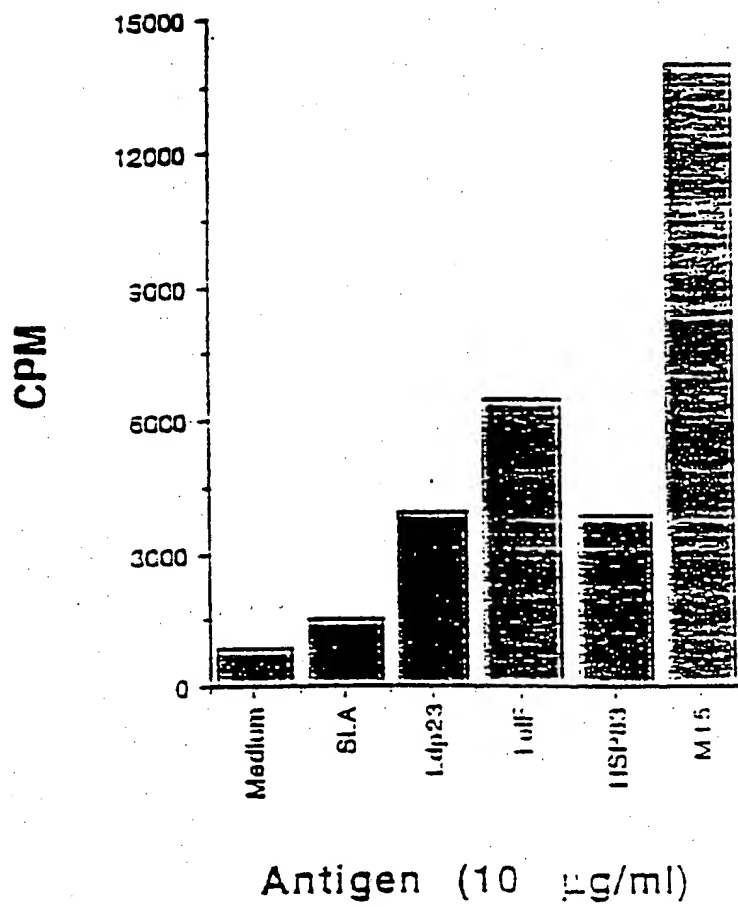


FIG. 16

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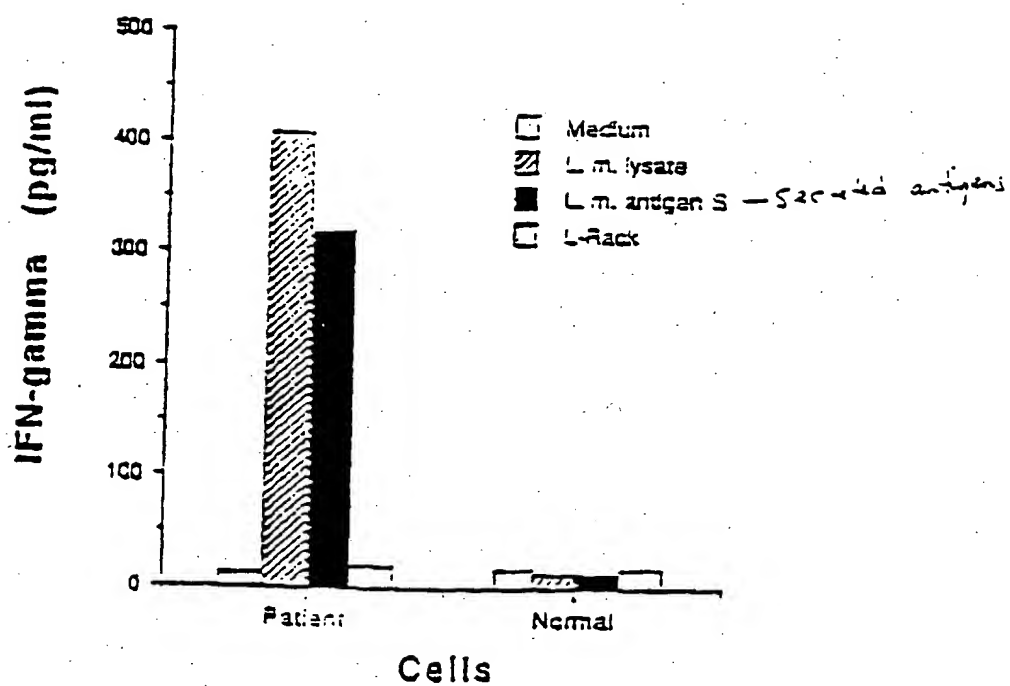


FIG. 15

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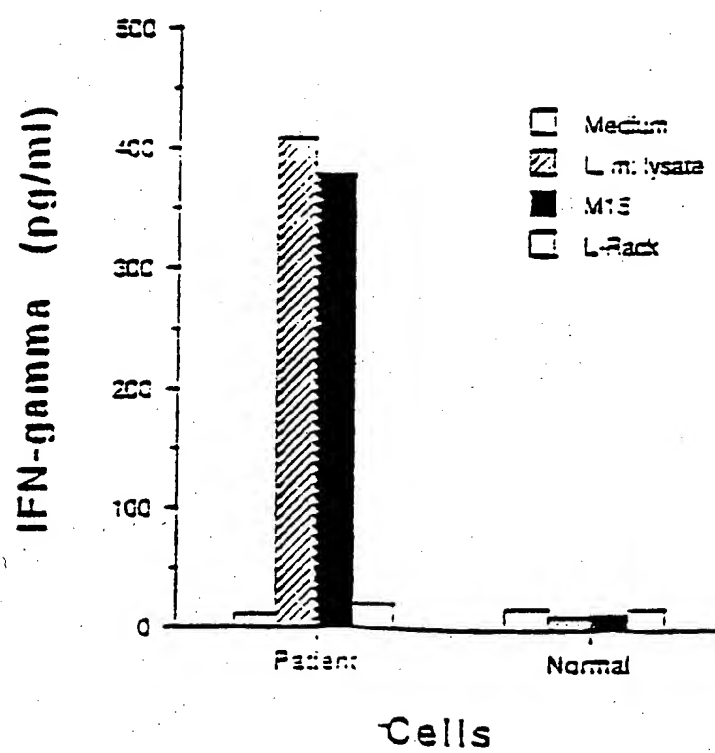


FIG. 14

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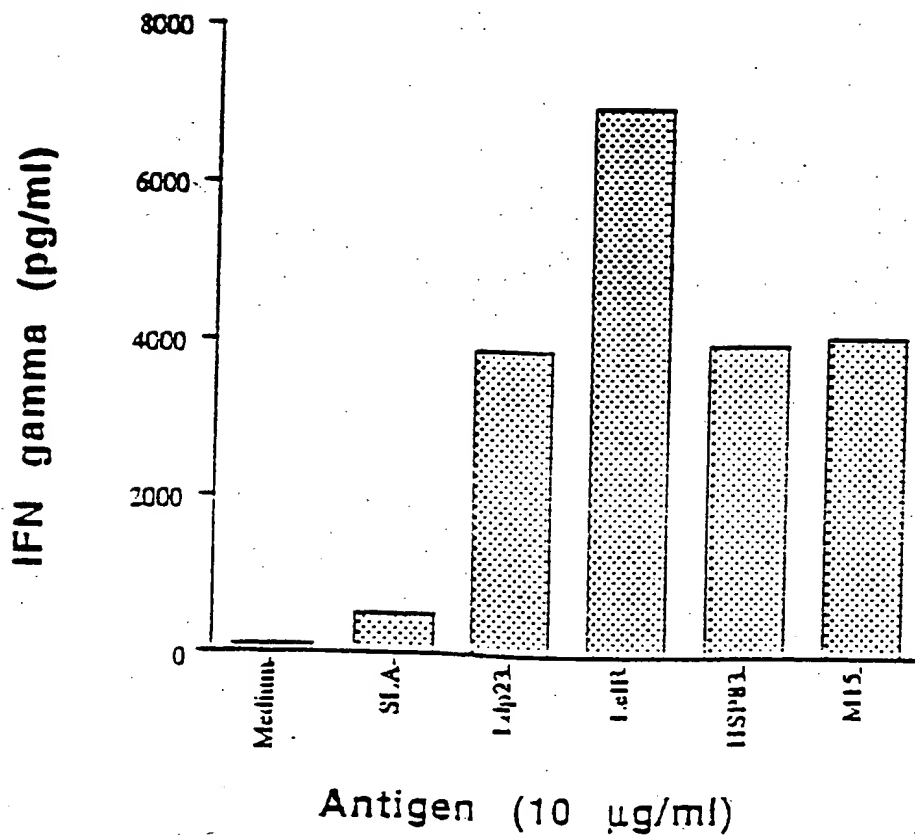
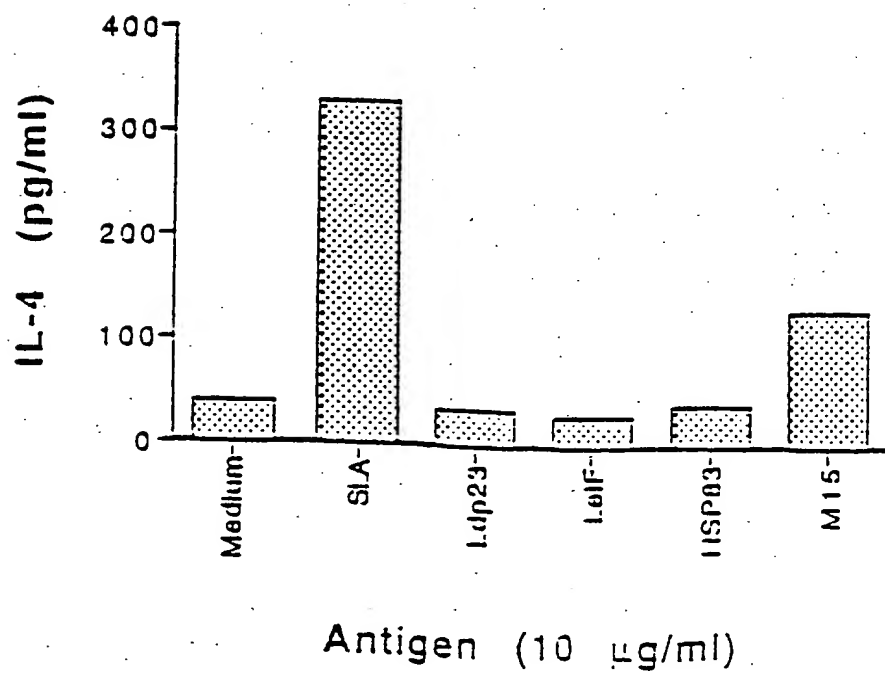


FIG. 13A



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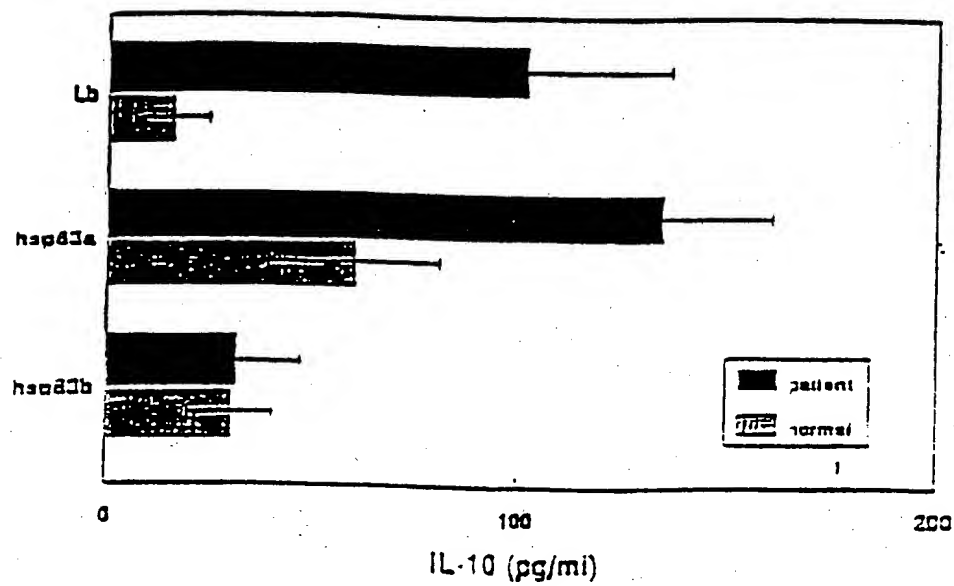


FIG. 11

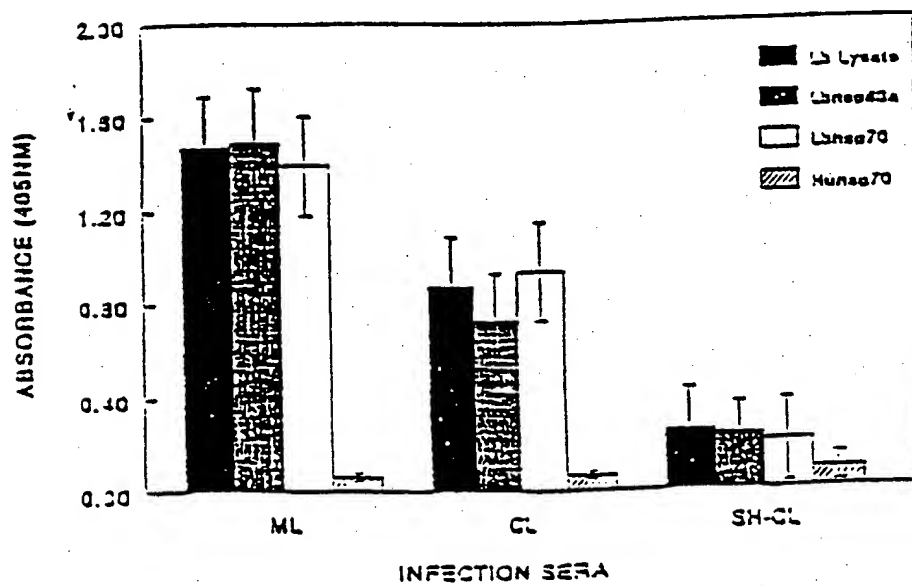
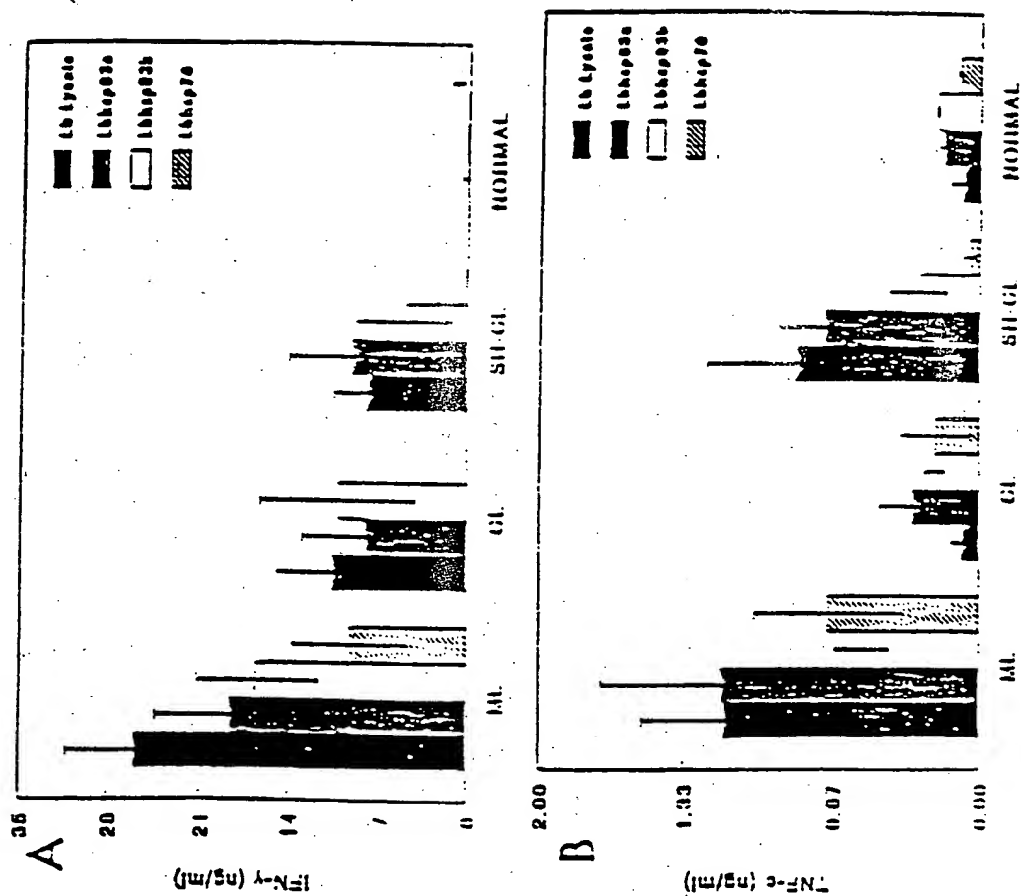


FIG. 12

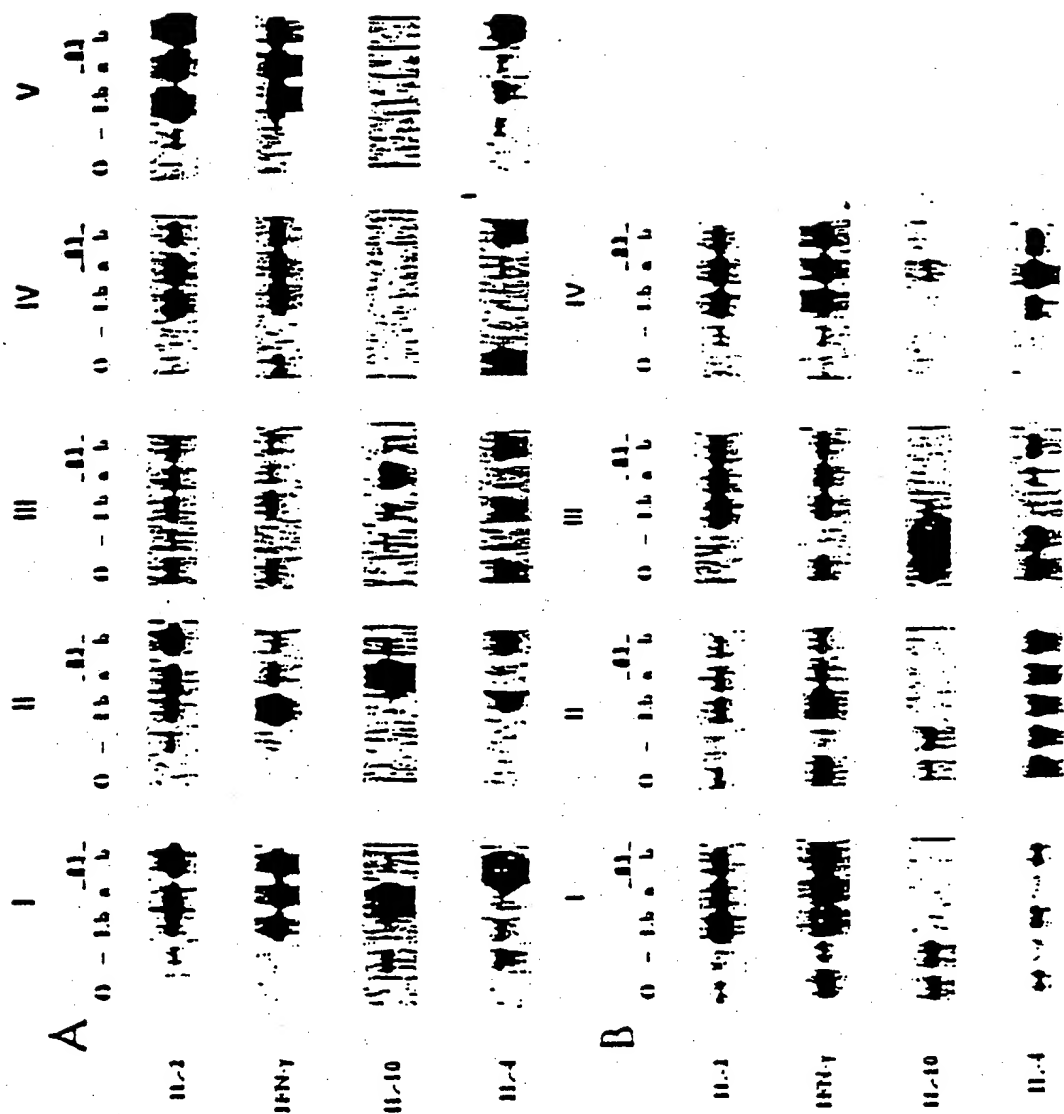
10/29

FIG. 10



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FIG. 9



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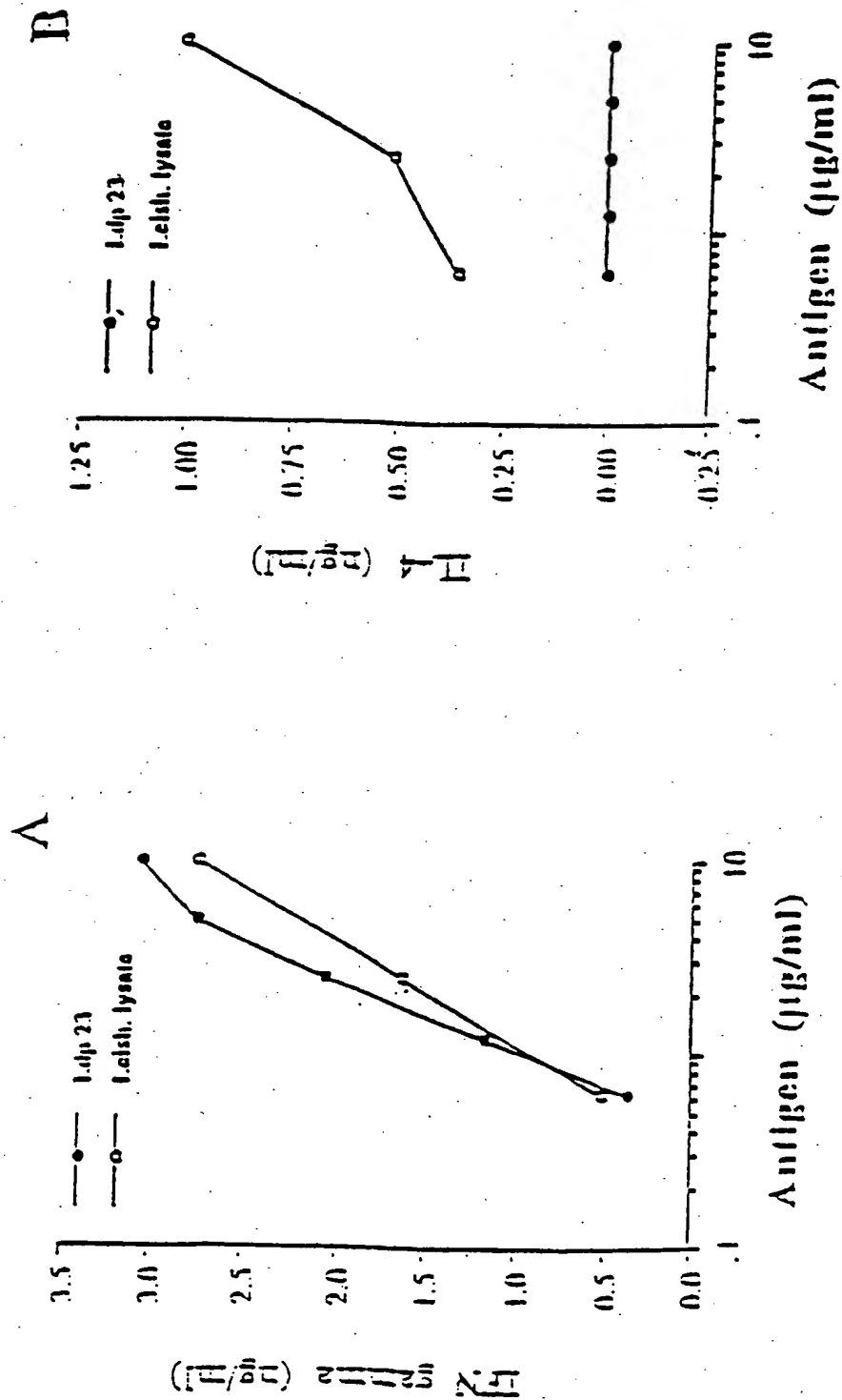


FIG. 8

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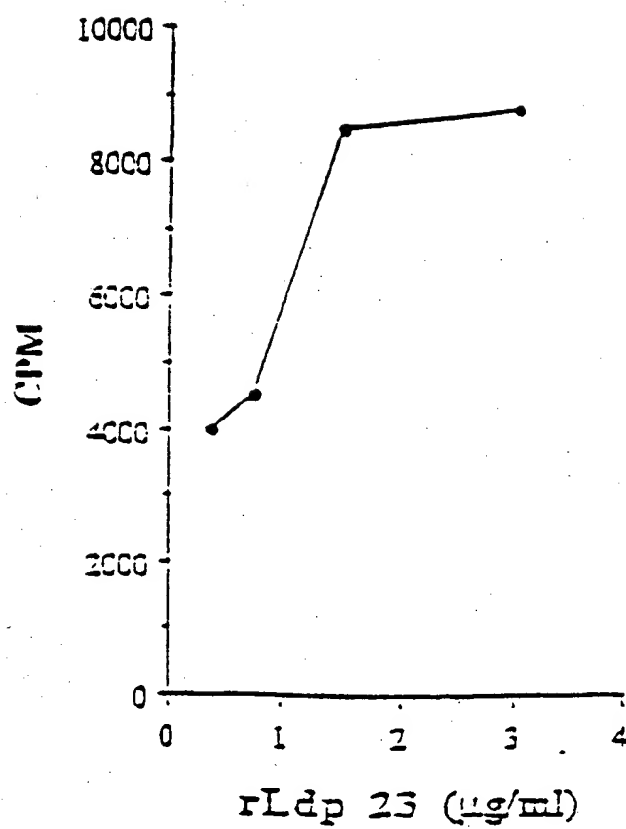


FIG. 7

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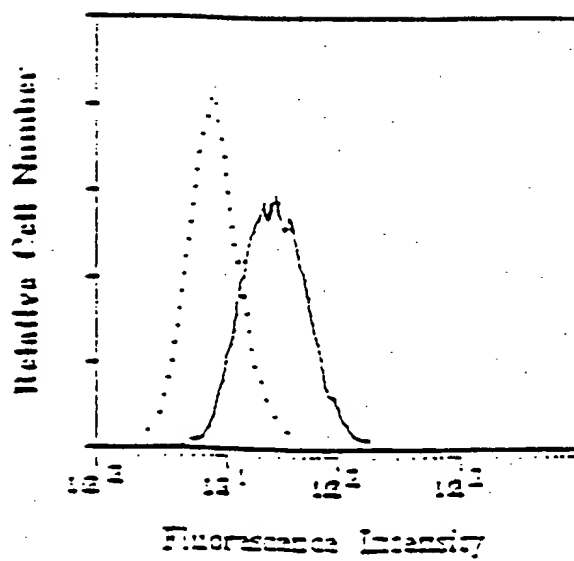


FIG. 6

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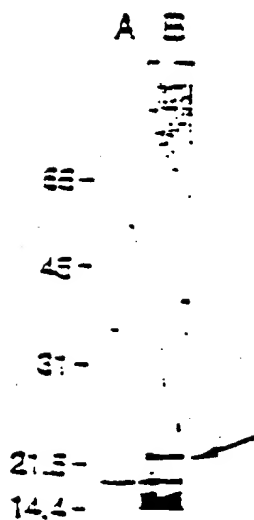


FIG. 5

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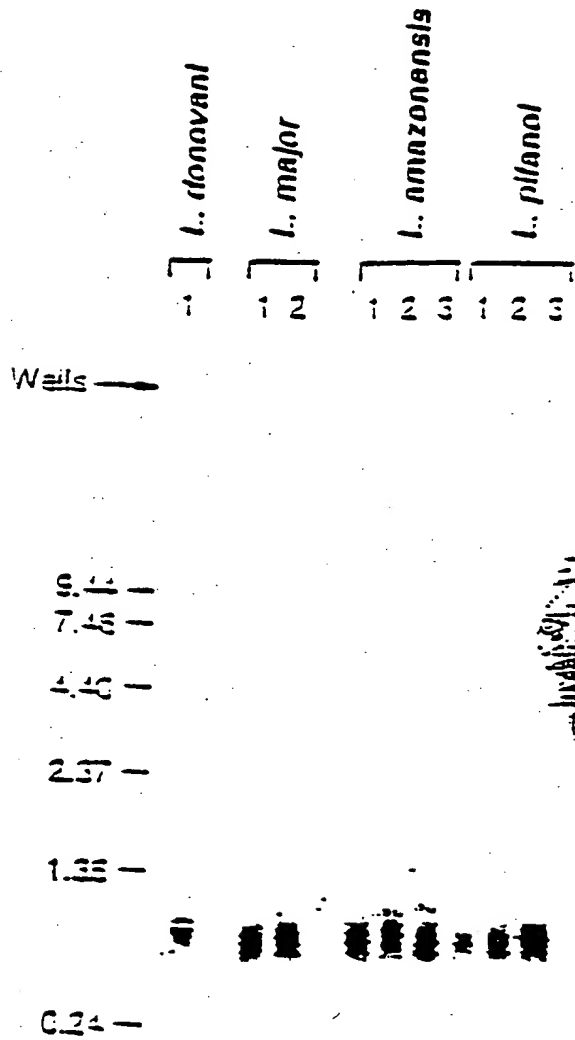


FIG. 4

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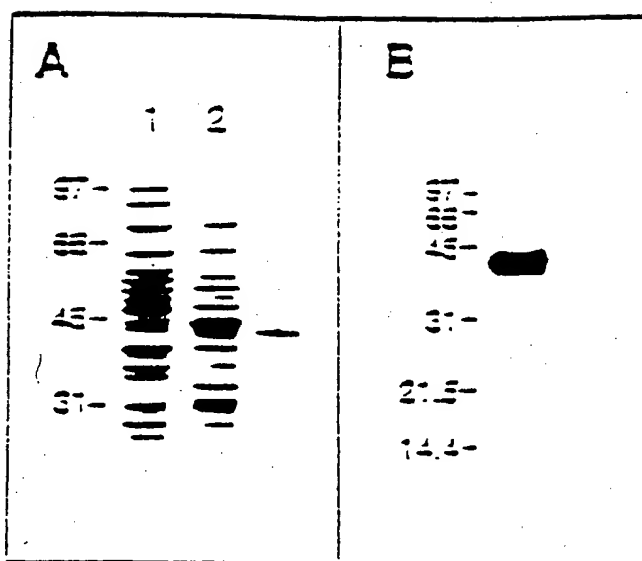


FIG. 3

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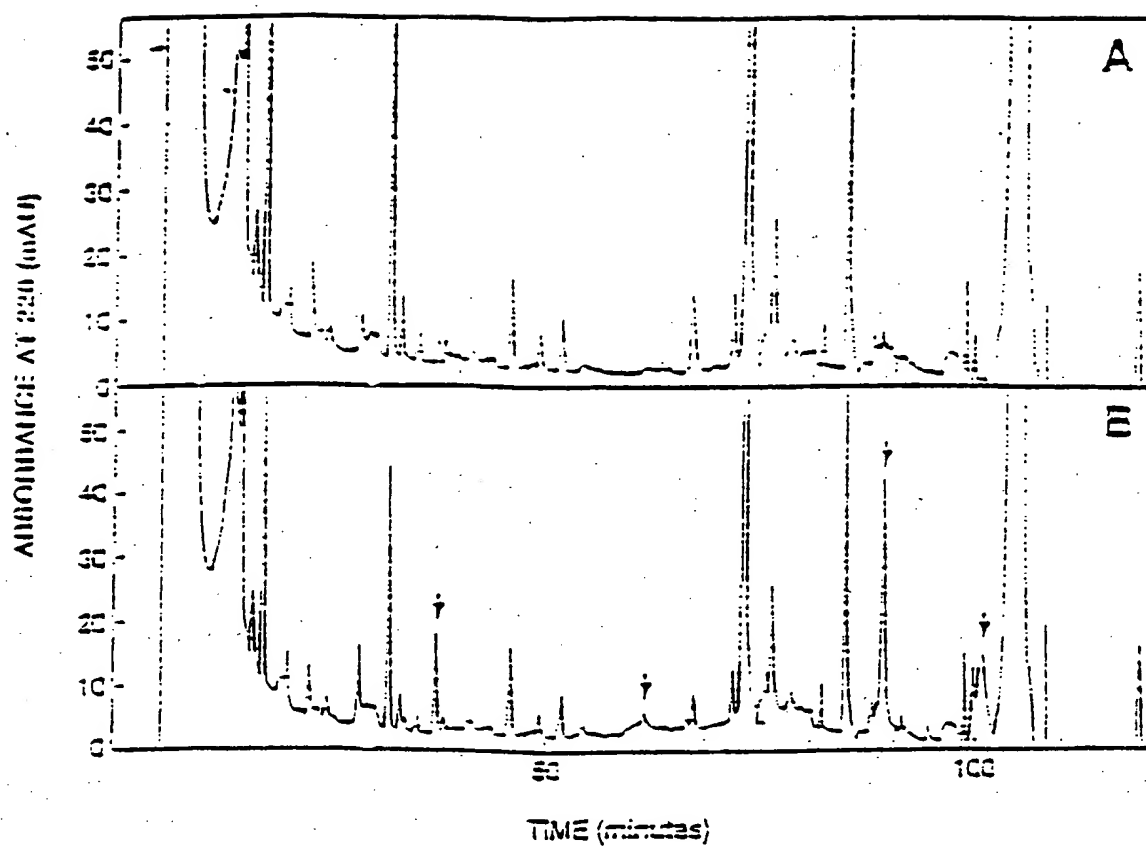


FIG. 2

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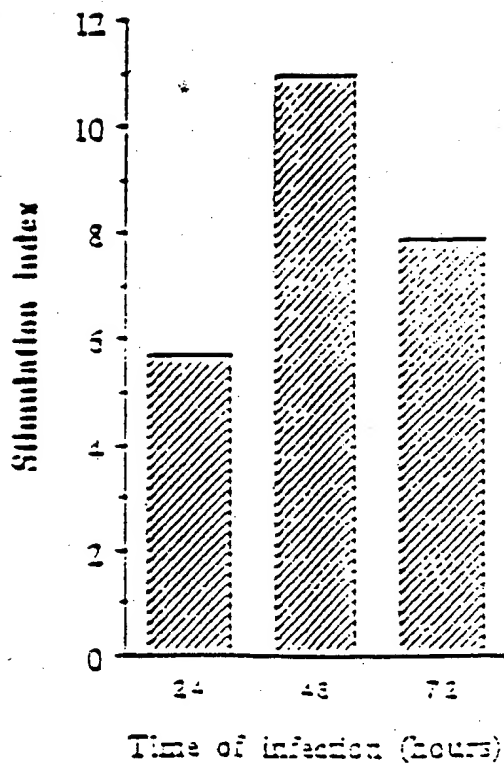


FIG. 1

63. A diagnostic kit comprising:
- (a) a pharmaceutical composition according to any one of claims 56 and 57; and
 - (b) apparatus sufficient to contact dermal cells of a patient with the pharmaceutical composition.

and/or modifications, wherein said antigen comprises an amino acid sequence selected from the group consisting of SEQ ID NO:39, 42, 55, 61, 62, 80, 81, and 83-87.

57. A pharmaceutical composition according to claim 56 further comprising a K39 *Leishmania* antigen.

58. A vaccine comprising a polypeptide and a non-specific immune response enhancer, the polypeptide comprising an immunogenic portion of a *Leishmania* antigen or a variant of said antigen that differs only in conservative substitutions and/or modifications, wherein said antigen comprises an amino acid sequence selected from the group consisting of SEQ ID NO:39, 42, 55, 61, 62, 80, 81 and 83-87.

59. A pharmaceutical composition according to any one of claims 56 and 57, for use in the manufacture of a medicament for inducing protective immunity against leishmaniasis in a patient.

60. A vaccine according to claim 58, for use in the manufacture of a medicament for inducing protective immunity against leishmaniasis in a patient.

61. A pharmaceutical composition according to any one of claims 56 or 57, for use in a method for detecting *Leishmania* infection in a patient.

62. A pharmaceutical composition according to claim 56, and a composition comprising a K39 *Leishmania* antigen, for use in a method for detecting *Leishmania* infection in a patient.

48. A pharmaceutical composition according to any one of claims 25, 26, 27, 29 and 30, for use in the manufacture of a medicament for stimulating a cellular and/or humoral immune response in a patient.

49. The composition of claim 48 wherein said response is a Th1 immune response.

50. The composition of claim 48 wherein said response is IL-12 production.

51. A vaccine according to any of claims 31-34, 40 and 41, for use in the manufacture of a medicament for stimulating a cellular and/or humoral immune response in a patient.

52. The vaccine of claim 51 wherein said response is a Th1 response.

53. The method of claim 51 wherein said response is IL-12 production.

54. A pharmaceutical composition according to any one of claims 25, 26, 27, 29 and 30, for use in the manufacture of a medicament for treating a patient afflicted with a disease responsive to IL-12 stimulation.

55. A vaccine according to any one of claims 31-34, 40 and 41, for use in the manufacture of a medicament for treating a patient afflicted with a disease responsive to IL-12 stimulation.

56. A pharmaceutical composition comprising a polypeptide and a physiologically acceptable carrier, the polypeptide comprising an immunogenic portion of a *Leishmania* antigen or a variant of said antigen that differs only in conservative substitutions

41. A vaccine comprising a DNA molecule according to claim 21.

42. A pharmaceutical composition according to any one of claims 25, 26, 27, 29 and 30, for use in the manufacture of a medicament for inducing protective immunity against leishmaniasis in a patient.

43. The composition of claim 42 wherein the leishmaniasis is caused by a *Leishmania* species selected from the group consisting of *L. donovani*, *L. chagasi*, *L. infantum*, *L. major*, *L. amazonensis*, *L. braziliensis*, *L. panamensis*, *L. tropica* and *L. guyanensis*.

44. A vaccine according to any one of claims 31-34, 40 and 41, for use in a method for inducing protective immunity against leishmaniasis in a patient comprising administering.

45. The vaccine of claim 44 wherein the leishmaniasis is caused by a *Leishmania* species selected from the group consisting of *L. donovani*, *L. chagasi*, *L. infantum*, *L. major*, *L. amazonensis*, *L. braziliensis*, *L. panamensis*, *L. tropica* and *L. guyanensis*.

46. A pharmaceutical composition according to any one of claims 25, 26, 27, 29 and 30, for use in a method for detecting *Leishmania* infection in a patient.

47. A diagnostic kit comprising:

- (a) a pharmaceutical composition according to any one of claims 25, 26, 27, 29 and 30; and
- (b) apparatus sufficient to contact dermal cells of a patient with the pharmaceutical composition.

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(h) a polypeptide comprising an immunogenic portion of a *Leishmania* antigen having the amino acid sequence recited in SEQ ID NO:10, or a variant of said antigen that differs only in conservative substitutions and/or modifications;
and a non-specific immune response enhancer.

34. A vaccine comprising soluble *Leishmania* antigens and a non-specific immune response enhancer.

35. A vaccine according to any one of claims 31, 32, 33 and 34 wherein the non-specific immune response enhancer is an immunogenic portion of a *Leishmania* antigen having the amino acid sequence recited in SEQ ID NO:10, or a variant of said antigen that differs only in conservative substitutions and/or modifications.

36. A vaccine according to any one of claims 31, 32 and 33, further comprising soluble *Leishmania* antigens.

37. A vaccine according to claim 36 wherein the non-specific immune response enhancer is an immunogenic portion of a *Leishmania* antigen having the amino acid sequence recited in SEQ ID NO:10, or a variant of said antigen that differs only in conservative substitutions and/or modifications.

38. A vaccine according to any one of claims 31, 32, 33 and 34, further comprising a delivery vehicle.

39. The vaccine of claim 38 wherein the delivery vehicle is a biodegradable microsphere.

40. A vaccine comprising a DNA molecule according to any one of claims 3, 9 and 16.

28. A pharmaceutical composition according to any one of claims 25, 26 and 27, further comprising soluble *Leishmania* antigens.

29. A pharmaceutical composition according to any one of claims 25, 26 and 27, further comprising a K39 *Leishmania* antigen.

30. A pharmaceutical composition comprising soluble *Leishmania* antigens and a physiologically acceptable carrier.

31. A vaccine comprising a polypeptide according to any one of claims 1, 7, 13 and 15, and a non-specific immune response enhancer.

32. A vaccine comprising a polypeptide according to claim 20 and a non-specific immune response enhancer.

33. A vaccine comprising at least two different polypeptides selected from the group consisting of:

- (a) a polypeptide according to claim 1;
- (b) a polypeptide according to claim 7;
- (c) a polypeptide according to claim 13;
- (d) a polypeptide according to claim 15
- (e) a polypeptide according to claim 21;
- (f) a polypeptide comprising an immunogenic portion of a *Leishmania* antigen having the amino acid sequence recited in SEQ ID NO:6, or a variant of said antigen that differs only in conservative substitutions and/or modifications;
- (g) a polypeptide comprising an immunogenic portion of a *Leishmania* antigen having the amino acid sequence recited in SEQ ID NO:8, or a variant of said antigen that differs only in conservative substitutions and/or modifications; and

23. An expression vector comprising the DNA molecule of claim 21.
24. A host cell transformed or transfected with the expression vector of claim 23.
25. A pharmaceutical composition comprising a polypeptide according to any one of claims 1, 7, 13 and 15, and a physiologically acceptable carrier.
26. A pharmaceutical composition comprising a polypeptide according to claim 20 and a physiologically acceptable carrier.
27. A pharmaceutical composition comprising at least two different polypeptides selected from the group consisting of:
 - (a) a polypeptide according to claim 1;
 - (b) a polypeptide according to claim 7;
 - (c) a polypeptide according to claim 13
 - (d) a polypeptide according to claim 15;
 - (e) a polypeptide according to claim 20;
 - (f) a polypeptide comprising an immunogenic portion of a *Leishmania* antigen having the amino acid sequence recited in SEQ ID NO:6, or a variant of said antigen that differs only in conservative substitutions and/or modifications;
 - (g) a polypeptide comprising an immunogenic portion of a *Leishmania* antigen having the amino acid sequence recited in SEQ ID NO:8, or a variant of said antigen that differs only in conservative substitutions and/or modifications; and
 - (h) a polypeptide comprising an immunogenic portion of a *Leishmania* antigen having the amino acid sequence recited in SEQ ID NO:10, or a variant of said antigen that differs only in conservative substitutions and/or modifications;and a physiologically acceptable carrier.

16. An isolated DNA molecule comprising a nucleotide sequence encoding a polypeptide according to claims 13 or 15.

17. The DNA molecule of claim 16 wherein the nucleotide sequence is selected from the group consisting of:

(a) sequences recited in SEQ ID NO: 21, 23, 25, 29-31, 34, 45-48 and 74; and

(b) DNA sequences that hybridize to a nucleotide sequence complementary to a sequence recited in SEQ ID NO: 21, 23, 25, 29-31, 34, 45-48 and 74 under moderately stringent conditions.

18. An expression vector comprising the DNA molecule of claim 16.

19. A host cell transformed or transfected with the expression vector of claim 18.

20. A polypeptide comprising an immunogenic portion of a *Leishmania* antigen or a variant of said antigen that differs only in conservative substitutions and/or modifications, wherein said antigen comprises an amino acid sequence provided in SEQ ID NO: 20.

21. An isolated DNA molecule comprising a nucleotide sequence encoding a polypeptide according to claim 20.

22. The DNA molecule of claim 21 wherein the nucleotide sequence is selected from the group consisting of:

(a) a sequence recited in SEQ ID NO: 19; and

(b) DNA sequences that hybridize to a nucleotide sequence complementary to the sequence of SEQ ID NO: 19 under moderately stringent conditions.

8. The polypeptide of claim 7, comprising amino acids 1-175 of SEQ ID NO:4.
9. An isolated DNA molecule comprising a nucleotide sequence encoding a polypeptide according to claim 7.
10. The DNA molecule of claim 9 wherein the nucleotide sequence is selected from the group consisting of:
- (a) nucleotides 25 through 549 of SEQ ID NO:3; and
 - (b) DNA sequences that hybridize to a nucleotide sequence complementary to nucleotides 25 through 549 of SEQ ID NO:3 under moderately stringent conditions.
11. A recombinant expression vector comprising the DNA molecule of claim 9.
12. A host cell transformed or transfected with the expression vector of claim 11.
13. A polypeptide comprising an immunogenic portion of a *Leishmania* antigen or a variant of said antigen that differs only in conservative substitutions and/or modifications, wherein said antigen comprises an amino acid sequence selected from the group consisting of sequences recited in SEQ ID NO: 22, 24, 26, 36-38, 41, 49-53 and 82.
14. An antigenic epitope of a *Leishmania* antigen comprising an amino acid sequence recited in SEQ ID NO:43.
15. A polypeptide comprising at least two contiguous antigenic epitopes according to claim 14.

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CLAIMS

1. A polypeptide comprising an immunogenic portion of a *Leishmania* antigen having the amino acid sequence recited in SEQ ID NO:2, or a variant of said antigen that differs only in conservative substitutions and/or modifications.
2. The polypeptide of claim 1, comprising amino acids 1 - 564 of SEQ ID NO:2.
3. An isolated DNA molecule comprising a nucleotide sequence encoding a polypeptide according to claim 1.
4. The DNA molecule of claim 3 wherein the nucleotide sequence is selected from the group consisting of:
 - (a) nucleotides 421 through 2058 of SEQ ID NO:1; and
 - (b) DNA sequences that hybridize to a nucleotide sequence complementary to nucleotides 421 through 2058 of SEQ ID NO:1 under moderately stringent conditions.
5. A recombinant expression vector comprising the DNA molecule of claim 3.
6. A host cell transformed or transfected with the expression vector of claim 5.
7. A polypeptide comprising an immunogenic portion of a *Leishmania* antigen having the amino acid sequence recited in SEQ ID NO:4, or a variant of said antigen that differs only in conservative substitutions and/or modifications.

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[illegible]

(2) INFORMATION FOR SEQ ID NO:87:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 69 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

[illegible]

153

Gly Leu Lys Arg Leu Ala Lys Ser Asp Pro Leu Val Val Cys Ser Ile
 260 265 270
 Glu Glu Ser Gly Glu His Ile Val Ala Gly Ala Gly Glu Leu His Leu
 275 280 285
 Glu Ile Cys Leu Lys Asp Leu Gln Glu Asp Phe Met Asn Gly Ala Pro
 290 295 300
 Leu Lys Ile Ser Glu Pro Val Val Ser Phe Arg Glu Thr Val Thr Asp
 305 310 315 320
 Val Ser Ser Gln Gln Cys Leu Ser Lys Ser Ala Asn Lys His Asn Arg
 325 330 335
 Leu Phe Cys Arg Gly Ala Pro Leu Thr Glu Xaa Leu Ala Leu Ala Xaa
 340 345 350
 Xaa Glu Gly Thr Ala Gly Pro Xaa Ala
 355 360

(2) INFORMATION FOR SEQ ID NO:86:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 149 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

Arg Ile Asn Val Tyr Phe Asp Xaa Ser Thr Gly Gly Arg Tyr Val Pro
 1 5 10 15
 Arg Ala Val Leu Met Asp Leu Glu Pro Gly Thr Met Asp Ser Val Arg
 20 25 30
 Ala Gly Pro Tyr Gly Gln Leu Phe Arg Pro Asp Asn Phe Ile Phe Gly
 35 40 45
 Gln Ser Gly Ala Gly Asn Asn Trp Ala Lys Gly His Tyr Thr Glu Gly
 50 55 60
 Ala Glu Leu Ile Asp Ser Val Leu Asp Val Cys Arg Lys Glu Ala Glu
 65 70 75 80
 Ser Cys Asp Cys Leu Gln Gly Phe Gln Leu Ser His Ser Leu Gly Gly
 85 90 95
 Gly Thr Gly Ser Gly Met Gly Thr Leu Leu Ile Ser Xaa Leu Arg Xaa

152

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

Lys	Lys	Trp	Ile	Lys	Gln	Glu	Thr	Asn	Ala	Asp	Gly	Glu	Arg	Val	Arg	1	5	10	15
Arg	Ala	Phe	Cys	Gln	Phe	Cys	Leu	Asp	Pro	Ile	Tyr	Gln	Ile	Phe	Asp	20	25	30	
Ala	Val	Met	Asn	Glu	Lys	Lys	Asp	Lys	Val	Asp	Lys	Met	Leu	Lys	Ser	35	40	45	
Leu	His	Val	Thr	Leu	Thr	Ala	Glu	Glu	Arg	Glu	Gln	Val	Pro	Xaa	Lys	50	55	60	
Leu	Leu	Lys	Thr	Val	Met	Met	Xaa	Phe	Leu	Pro	Ala	Ala	Glu	Thr	Leu	65	70	75	80
Leu	Gln	Met	Ile	Val	Ala	His	Leu	Pro	Ser	Pro	Lys	Lys	Ala	Gln	Ala	85	90	95	
Tyr	Arg	Ala	Glu	Met	Leu	Tyr	Ser	Gly	Glu	Ala	Ser	Pro	Glu	Asp	Lys	100	105	110	
Tyr	Phe	Met	Gly	Ile	Lys	Asn	Cys	Asp	Pro	Ala	Ala	Pro	Leu	Met	Leu	115	120	125	
Tyr	Ile	Ser	Lys	Met	Val	Pro	Thr	Ala	Asp	Arg	Gly	Arg	Phe	Phe	Ala	130	135	140	
Phe	Gly	Arg	Ile	Phe	Ser	Gly	Lys	Val	Arg	Ser	Gly	Gln	Lys	Val	Arg	145	150	155	160
Ile	Met	Gly	Asn	Asn	Tyr	Val	Tyr	Gly	Lys	Lys	Gln	Asp	Leu	Tyr	Glu	165	170	175	
Asp	Lys	Pro	Val	Gln	Arg	Ser	Val	Leu	Met	Met	Gly	Arg	Tyr	Gln	Glu	180	185	190	
Ala	Val	Glu	Asp	Met	Pro	Cys	Gly	Asn	Val	Val	Gly	Leu	Val	Gly	Val	195	200	205	
Asp	Lys	Tyr	Ile	Val	Lys	Ser	Ala	Thr	Ile	Thr	Asp	Asp	Gly	Glu	Ser	210	215	220	
Pro	His	Pro	Leu	Arg	Asp	Met	Lys	Tyr	Ser	Val	Ser	Pro	Val	Val	Arg	225	230	235	240
Val	Ala	Val	Glu	Ala	Lys	Asn	Pro	Ser	Asp	Leu	Pro	Lys	Leu	Val	Glu	245	250	255	

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- (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

```

Leu Thr Glu Phe Gln Thr Asn Leu Val Pro Tyr Pro Arg Ile His Phe
1           5           10           15

Val Leu Thr Ser Tyr Ala Pro Val Val Ser Ala Glu Lys Ala Tyr His
          20           25           30

Glu Gln Leu Ser Val Ala Asp Ile Thr Asn Ser Val Phe Glu Pro Ala
          35           40           45

Gly Met Leu Thr Lys Cys Asp Pro Arg His Gly Lys Tyr Met Ser Cys
          50           55           60

Cys Leu Met Tyr Arg Gly Asp Val Val Pro Lys Asp Val Asn Ala Ala
          65           70           75           80

Ile Ala Thr Ile Lys Thr Lys Arg Thr Ile Gln Phe Val Asp Trp Cys
          85           90           95

Pro Thr Gly Phe Lys Cys Gly Ile Asn Tyr Gln Pro Pro Thr Val Val
          100          105          110

Pro Gly Gly Asp Leu Ala Lys Val Gln Arg Ala Val Cys Met Ile Ala
          115          120          125

Asn Ser Thr Ala Ile Ala Glu Val Phe Ala Arg Ile Asp His Lys Phe
          130          135          140

Asp Leu Met Tyr Ser Lys Arg Ala Phe Val His Trp Tyr Val Gly Glu
          145          150          155          160

Gly Met Glu Glu Gly Glu Phe Ser Glu Ala Arg Glu Asp Leu Ala Ala
          165          170          175

Leu Glu Lys Asp Tyr Glu Glu Val Gly Ala Glu Ser Ala Asp Asp Met
          180          185          190

Gly Glu Glu Asp Val Glu Glu Tyr
          195          200

```

(2) INFORMATION FOR SEQ ID NO:85:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 361 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS:

150

Leu Ser Lys Leu Thr Gly Val Phe Ala Pro Arg Pro Arg Pro Gly Pro
 20 25 30

His Lys Leu Arg Glu Cys Leu Pro Leu Leu Val Ile Ile Arg Asn Arg
 35 40 45

Leu Lys Tyr Ala Leu Asn Ala Arg Glu Gly Glu Met Ile Leu Arg Gln
 50 55 60

Gly Leu Val His Val Asp Asn His Pro Arg Arg Asp Gly Lys Tyr Pro
 65 70 75 80

Ala Gly Phe Met Asp Val Val Glu Ile Pro Lys Thr Gly Asp Arg Phe
 85 90 95

Arg Leu Met Tyr Asp Val Lys Gly Arg Phe Ala Leu Val Asn Leu Ser
 100 105 110

Glu Ala Glu Ala Gln Ile Lys Leu Met Lys Val Val Asn Leu Tyr Thr
 115 120 125

Ala Thr Gly Arg Val Pro Val Ala Val Thr His Asp Gly His Arg Ile
 130 135 140

Arg Tyr Pro Asp Pro His Thr Ser Ile Gly Asp Thr Ile Val Tyr Asn
 145 150 155 160

Val Lys Glu Lys Lys Cys Val Asp Leu Ile Lys Asn Arg Gln Gly Lys
 165 170 175

Ala Val Ile Val Thr Gly Gly Ala Asn Arg Gly Arg Ile Gly Glu Ile
 180 185 190

Val Lys Val Glu Cys His Pro Gly Ala Phe Asn Ile Ala His Leu Lys
 195 200 205

Asp Ala Ser Gly Ala Glu Phe Ala Thr Arg Ala Ala Asn Ile Phe Val
 210 215 220

Ile Gly Lys Asp Leu Asn Asn Leu Gln Val Thr Val Pro Lys Gln Gln
 225 230 235 240

Gly Leu Arg Met Asn Val Ile Gln Glu Arg Glu Glu Arg Leu Ile Ala
 245 250 255

Ala Glu Ala Arg Lys Asn Ala Pro Ala Arg Gly Ala Arg Arg Ala Arg
 260 265 270

Lys

(2) INFORMATION FOR SEQ ID NO:84:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 200 amino acids

149

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

Met	Ser	Ile	Ile	Lys	Glu	Asp	Asp	Ala	Val	Gly	Cys	Tyr	Met	Thr	Val	1	5	10	15
Thr	Leu	Val	Asp	Asp	Thr	Lys	Val	Glu	Gly	Thr	Ile	Phe	Thr	Tyr	Asn	20	25	30	
Ser	Lys	Glu	Gly	Ile	Ile	Val	Leu	Leu	Ser	Leu	Arg	Asp	Asp	Gln	Thr	35	40	45	
Asn	Met	Lys	Leu	Ile	Arg	Thr	Pro	Tyr	Ile	Lys	Asp	Phe	Ser	Leu	Ser	50	55	60	
His	Ala	Glu	Glu	Gly	Ala	His	Leu	Pro	Pro	Ala	Leu	Asp	Ser	Phe	Asn	65	70	75	80
Glu	Leu	Pro	Ser	Met	His	Ala	Gly	Arg	Asp	Lys	Ser	Ile	Phe	Lys	His	85	90	95	
Ala	Ser	Thr	Gln	Leu	Lys	Asn	Ala	Glu	Ala	Asn	Arg	Glu	Lys	His	Phe	100	105	110	
Asn	Ser	Val	Thr	Thr	Asp	Thr	Pro	Ile	Ala	Thr	Leu	Asp	Ala	Tyr	Leu	115	120	125	
Lys	Leu	Leu	Arg	Leu	Tyr	Pro	Leu	Ile	Glu	Trp	Asn	Ser	Asp	Glu	Gly	130	135	140	
Val	Ile	Gln	Val	Ser	Asp	Thr	Val	Ile	Val	Val	Gly	Asp	Pro	Asp	Trp	145	150	155	160
Arg	Thr	Pro	Lys	Ala	Met	Leu	Val	Asp	Gly	Ala	Pro	Glu	Lys	Asp	Arg	165	170	175	
Pro	Leu	Val	Asp	Arg	Leu	Gln	Val	Ala	Leu	Gly	Asn	Gly	Lys	Lys		180	185	190	

(2) INFORMATION FOR SEQ ID NO:83:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 273 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

Met	Ala	Lys	Lys	His	Leu	Lys	Arg	Leu	Tyr	Ala	Pro	Lys	Asp	Trp	Met	1	5	10	15
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	---	---	----	----

148

Gln Val Thr Asp Val Val Glu Lys Ala Ala Glu His Tyr Lys Val Thr
 165 170 175
 Pro Val Asp Gly Val Leu Ser His Met Met Lys Arg Tyr Ile Ile Asp
 180 185 190
 Xaa Tyr Arg Cys Ile Pro Gln Arg Arg Val Ala Glu His Met Val His
 195 200 205
 Asp Tyr Asp Leu Glu Lys Ala Gln Val Trp Thr Leu Asp Ile Val Met
 210 215 220
 Thr Ser Gly Lys Gly Lys Leu Lys Glu Arg Asp Ala Arg Pro Cys Val
 225 230 235 240
 Phe Lys Val Ala Leu Asp Ser Asn Tyr Ser Val Lys Met Glu Ser Ala
 245 250 255
 Lys Glu Val Gln Lys Glu Ile Asp Ser Xaa Tyr Ala Thr Phe Pro Phe
 260 265 270
 Ala Ile Arg Asn Leu Glu Ala Lys Lys Ala Arg Leu Gly Leu Asn Glu
 275 280 285
 Met Ala Lys His Gly Ala Val Ile Pro Tyr Pro Ile Leu Phe Glu Lys
 290 295 300
 Glu Gly Glu Val Val Ala His Phe Lys Ile Thr Val Leu Ile Ser Asn
 305 310 315 320
 Lys Lys Ile Glu Pro Ile Thr Gly Leu Lys Pro Gln Lys Ala Pro Ala
 325 330 335
 Leu Glu Pro Tyr Thr Asp Glu Met Leu Leu Ala Thr Asn Lys Leu Phe
 340 345 350
 Ala Val Ala Arg Glu Glu Gly Gly Glu Val Asp Gly Arg Gly Ile Arg
 355 360 365
 Asp Ala Val Leu Arg Ala Phe Val Gly Val Arg Leu Leu
 370 375 380

(2) INFORMATION FOR SEQ ID NO:82:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 191 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

147

Tyr Val Asn Asp Val Met Glu Arg Ile Cys Thr Glu Ala Ala Ser Ile
 50 55 60

Val Arg Ala Asn Lys Lys Arg Thr Leu Gly Ala Arg Glu Val Gln Thr
 65 70 75 80

Ala Val Arg Ile Val Leu Pro Ala Glu Leu Ala Lys His Ala Met Ala
 85 90 95

Glu Gly Thr Lys Ala Val Ser Ser Ala Ser Arg
 100 105

(2) INFORMATION FOR SEQ ID NO:81:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 381 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

Asp Glu Glu Glu Glu Asp Thr Thr Ile Asn Asn Ser Asp Val Val Val
 1 5 10 15

Arg Tyr Lys Lys Ala Ala Thr Trp Cys Asn Glu Thr Leu Arg Val Leu
 20 25 30

Ile Asp Ala Thr Lys Pro Gly Ala Lys Val Cys Asp Leu Cys Arg Leu
 35 40 45

Gly Asp Asp Thr Ile Thr Ala Xaa Val Lys Thr Met Phe Lys Gly Thr
 50 55 60

Glu Lys Gly Ile Ala Phe Pro Thr Cys Ile Ser Val Asn Asn Cys Val
 65 70 75 80

Cys His Asn Ser Pro Gly Val Ser Asp Glu Thr Thr Gln Gln Glu Ile
 85 90 95

Ala Met Gly Asp Val Val His Tyr Asp Leu Gly Ile His Val Asp Gly
 100 105 110

Tyr Cys Ala Val Val Ala His Thr Ile Gln Val Thr Glu Asp Asn Glu
 115 120 125

Leu Gly Lys Asp Glu Lys Ala Ala Arg Val Ile Thr Ala Ala Tyr Asn
 130 135 140

Ile Leu Asn Thr Ala Leu Arg Gln Met Arg Pro Gly Thr Thr Ile Tyr
 145 150 155 160

146

ACCTTCTCCG TCATCCCGTC CCCCCGCGTG TCGGATACCG TTGTGGANCC GTACAACACG 420

ACCCTCTCTG TGCACCAGCT CGTGGAA 447

(2) INFORMATION FOR SEQ ID NO:79:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 375 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

GTAACCCGCT GGTGTACGCA TATGTAGACA CAGACGGGCA GCACGAGACG ACGTTCCTCG 60
 CGATCCCTGT GGTGCTTGGC ATGAATGGAA TCGAGAAGCG CCTGCCGATT GGTCCGCTGC 120
 ACTCGACGGA GGAAACGCTG CTGAAGGCGG CACTGCCGGT GATCAAGAAG AATATCGTGA 180
 AGGGCAGCGA GTTCGCGCGC TCACACCTGT AGCACCTCAG CTTTTTTTTT TTGCGTTAAA 240
 CGGGCGTGGG AAGCACCTCG ATACTTCGCT TCGCGCTGAC GGACCCGCAC GACATCGTTC 300
 GTCATCCCCC TCCCCCTCTT CGGCCCTATA CGCATGAAGG AGTGAATTA TGCAACAGCA 360
 TGTNATATC AAGTG 375

(2) INFORMATION FOR SEQ ID NO:80:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 107 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

Met Ala Ser Ser Arg Lys Ala Ser Asn Pro His Lys Ser His Arg Lys
 1 5 10 15
 Pro Lys Arg Ser Trp Asn Val Tyr Val Gly Arg Ser Leu Lys Ala Ile
 20 25 30
 Asn Ala Gln Met Ser Met Ser His Arg Thr Met Lys Ile Val Asn Ser
 35 40 45

145

GATGCTGTAC TCTGGCGAGG CGTCGCCGGA GGACAAGTAC TTCATGGGTA TCAAGAACTG	360
CGACCCCGCT GCGCCGCTCA TGCTGTACAT CAGCAAGATG GTGCCGACGG CCGACCGCGG	420
CCGCTTCTTC GCCTTTGGCC GCATCTTCTC CGGTAAGGTG CGCAGCGGCC AGAAGGTGCG	480
CATCATGGGT AACAACTACG TCTACGGCAA GAAGCAGGAC CTGTACGAGG ACAAGCCTGT	540
GCAGCGCTCC GTGCTGATGA TGGGCCGCTA CCAGGAGGCC GTGGAGGACA TGCCGTGCGG	600
TAACGTGGTG GGCCTTGTGG GCGTGGACAA GTACATCGTG AAGTCCGCGA CGATCACGGA	660
CGATGGCGAG AGCCCGCACC CGCTGCGCGA CATGAAGTAC TCTGTGTCGC CCGTCGTGCG	720
TGTGGCCCTG GAGGCGAAGA ACCCGTCCGA CCTGCCGAAG CTTGTGGAGG GCCTGAAGCG	780
CCTTGCCAAG TCCGACCCGC TGGTGGTGTG CAGCATTGAG GAGTCTGGCG AGCACATTGT	840
TGCCGGCGCT GGCAGCTTC ACCTTGAGAT TTGCCTGAAG GATCTCCAGG AGGACTTCAT	900
GAACGGCGCG CCGCTNAAGA TCTCCGAGCC GGTGGTGTG TTCCGCGAGA CCGTGACGGA	960
TGTGTCGTG CAGCAGTGCC TGTCGAAGTC TGCGAACAAG CACAACCGTC TCTTCTGCCG	1020
CGGTGCGCCG CTNACAGAGG ANCTGGCGCT GGCATNGAN GAAGGCACCG CTGGTCCCGA	1080
NGCGGA	1086

(2) INFORMATION FOR SEQ ID NO:78:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 447 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

CGCATCAACG TCTACTTCGA TNAGTCGACG GGAGGCCGCT ACGTGCCGCG CGCCGTGCTG	60
ATGGACCTCG AGCCCGGCAC TATGGACTCC GTTCGCGCCG GCCCGTACGG CCAGCTGTTC	120
CGCCCGGACA ACTTCATCTT TGGTCAGTCC GGCGCTGGCA ACAACTGGGC CAAGGGCCAC	180
TACTGAGG GCGCGGAGCT GATCGACTCC GTGCTTGATG TGTGCCGCAA GGAGGCGGAG	240
AGCTGCGACT GCCTGCAGGG CTTCCAGCTG TCTCACTCCC TCGGCGGCGG CACGGGCTCC	300
GGCATGGGCA CGCTGCTCAT TTCCAANCTG CGCGANGAGT ACCCGGACCG GATCATGATG	360

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(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

CTGACGGAGT TCCAGACGAA CCTTGTGCCG TACCCGCGCA TCCACTTCGT GCTGACAAGC	60
TACGCTCCGG TGGTGTCTGC CGAGAAGGCG TACCACGAGC AGCTNTCCGT CGCGGACATC	120
ACGAACTCGG TTTTGTAGCC TGCTGGCATG CTNACAAAGT GCGATCCTCG CCACGGCAAG	180
TACATGTCGT GCTGCCTCAT GTACCGCGGT GATGTCTGTC CGAAGGATGT CAACGCCGCG	240
ATTGCGACGA TCAAGACGAA GCGCACAATT CAGTTCGTGG ACTGGTGCCC GACCGGCTTC	300
AAGTGCGGCA TCAACTACCA GCCGCCGACC GTTGTGCCCC GCGGTGACCT CGCGAAGGTG	360
CAGCGCGCCG TGTGCATGAT TGCCAACTCG ACCGCGATCG CTGAGGTGTT TGCCCGCATC	420
GACCACAAGT TCGACCTGAT GTACAGCAAG CGCGCGTTTG TGCACTGGTA CGTGGGTGAG	480
GGCATGGAGG AGGGCGAGTT CTCCGAGGCG CGCGAGGATC TCGCTGCGCT GGAGAAGGAC	540
TACGAGGAGG TTGGCGCCGA GTCCGCCGAC GACATGGGCG AGGAGGACGT CGAGGAGTAC	600
TAAGGTAGAC TCGTGCCGCG CGCTGATGAT GTAGGTGCAC GCGTGCGTGT GCTGCAGCGG	660
AGCCGCCGCC ACCGCGACTG TGTGTGTGTG CGCGCGTGAC GACCGGCTCG AG	712

(2) INFORMATION FOR SEQ ID NO:77:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1086 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

CAAGAAGTGG ATCAAGCAGG AGACGAACGC CGATGGCGAG CGCGTGCGCC GCGCGTTCTG	60
CCAGTTCTGC CTAGACCCCA TCTACCAGAT CTTGACGCT GTGATGAACG AGAAGAAGGA	120
CAAGGTGGAC AAGATGCTCA AGTCGCTGCA CGTGACGCTN ACGGCTGAGG AGCGCGAGCA	180
GGTGCCGAAN AAGCTTCTGA AGACGGTGAT GATGAANTTC CTGCCGGCTG CTGAGACGCT	240
GCTACAGATG ATCGTGGCGC ACCTGCCGTC GCCCAAGAAG GCGCAGGCGT ACCGTGCGGA	300

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

TTTCTGTACT TTATTGAACA TCAGTAGAAC ACGTTCTTCC CGCAAAGATG GCCAAGAAGC	60
ACCTCAAGCG CTTGTATGCG CCCAAGGACT GGATGCTGAG CAAGCTGACC GGC GTGTTTCG	120
CGCCGCGTCC GCGTCCGGGT CCGCACAAGC TGC GCGAGTG CCTGCCGCTN CTGGTGATCA	180
TCCGCAACCG GCTGAAGTAC GCGCTGAACG CGCGCGAGGG TGAGATGATC CTGCGCCAGG	240
GTCTGGTGCA CGTGGACAAC CACCCGCGCC GCGACGGCAA GTATCCCGCC GGTTCATGG	300
ACGTGGTCGA GATCCCGAAG ACGGGCGACC GCTTCCGCCT GATGTACGAC GTCAAGGGCC	360
GCTTCGCGTT GGTGAACCTG TCCGAGGCGG AGGCGCAGAT CAAGCTGATG AAGGTTGTGA	420
ACCTGTACAC GGCCACCGGC CGCGTGCCGG TCGCTGTGAC GCACGACGGC CACCGCATCC	480
GCTACCCGGA CCCGCACACC TCCATTGGTG ACACCATCGT GTACAACGTC AAGGAGAAGA	540
AGTGCGTGGA CCTGATCAAG AACCGCCAGG GCAAGGCCGT GATCGTGACC GGTGGCGCCA	600
ACCGCGGCCG CATCGGCGAG ATCGTGAAGG TGGAGTGCCA CCCC GG TGCG TTCAACATTG	660
CGCACCTGAA GGACGCGTCC GCGCCGAGT TCGCCACCCG CGCCGCGAAC ATCTTCGTGA	720
TCGGCAAGGA CCTGAACAAC CTGCAGGTAA CGGTGCCGAA GCAGCAGGGC CTGCGCATGA	780
ACGTGATCCA GGAGCGCGAG GAGCGCCTGA TCGCGGCGGA GGCCCGCAAG AACGCGCCGG	840
CTCGTGGTGC CCGCAGGGCC CGCAAGTGAG GAGGCGATTA CACGCATGCG TGT TGTGGC	900
TCTGAAGCGA CTTGGCGGGT CGGCTGTGAG GGT TGTGAG GAGGTGTGTG ATGCGTGTGA	960
AGTCCTTCTC CGTTCTCAGC TCTCTCTGTG CTGTAGCTGT GCCTTTCCCC AGATCGCTTT	1020
ACCGCATTTG CATACATCTG TGTAGTCGCA TGTGCGTGTT TCTGTCTCTC GGTGGGTCTC	1080
CCTCTCCCTC CCTTTCTGCC TCTCTCTTTG AGTGGGTGTG CATGCGTCGC GCGCGACGGG	1140
CTCCGCTTNA GTGATTCTCT CGTGTTTIAN GGCTGTTTTY TTTCTYAGTT NAGCGTTTTY	1200
GTTTCATGATT TCCTCAGACC CAAAAAAAAA AAAAAAAA	1238

(2) INFORMATION FOR SEQ ID NO:76:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 712 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single

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TGTGTGTGTG TGGGGGGGCG TGTTACGAGT ACAAAGAGG CTCGATCTTT GCGATCTTTT 1380
 CTTTCTGTAA ACAGGAACAT AAGTAACCAA AAAAAAAAAA AAAAAACTCG AG 1432

(2) INFORMATION FOR SEQ ID NO:74:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 873 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

CTTTATTGTC ATCACTGTAA AGCACTGTTT TTTCTTTCAC TTTTCTTGA GTGTTTTCTT 60
 CTATTCACCA TGAGCATTAT CAAGGAGGAC GACGCCGTGG GCTGCTACAT GACGGTGACC 120
 CTCGTGGACG ACACCAAGGT GGAGGGTACC ATCTTCACCT ACAATTCCAA GGAGGGCATC 180
 ATAGTACTCC TGTCCCTCCG CGACGATCAG ACGAACATGA AGCTAATCCG CACTCCGTAC 240
 ATCAAAGACT TCAGCCTTTC ACACGCTGAG GAGGGAGCGC ACCTGCCCCC GGCACCTGGAC 300
 TCCTTCAACG AGCTTCCGTC CATGCACGCC GGCCGCGACA AGTCCATCTT CAAGCACGCC 360
 AGCACGCAGC TCAAGAACGC CGAGGCGAAC CGCGAAAAGC ACTTCAACTC TGTCACGACC 420
 GACACACCGA TTGCCACACT TGATGCGTAC CTCAAGCTCC TGCGGCTATA CCCCTTAATT 480
 GAGTGGAACA GCGACGAGGG TGTCATCCAG GTCTCGGACA CCGTCATTGT CGTAGGAGAC 540
 CCCGACTGGC GGACGCCCAA GGCAATGCTG GTGGACGGCG CCCCTGAGAA GGACAGACCG 600
 CTTGTAGATC GCCTGCAGGT TCGCTCGGM AACGGCAAGA AGTGATTCAG TGTGTAGCGG 660
 ACAGAACATC GTGTGCTTGT GTGTCTGTTT GANGTTTGTT TGTCTTCTCT TTGTGGTACT 720
 GCGTACGACG GCGCCTTCTC CCGGTGGTGG GTGAGTCCAT AAGCAGTTGA GTTCTYGGTT 780
 GTAGNAAVGC CTYACYGCCG ACCATATGGG AGAGGGCGAA CAAATNTTTG ATAGAAGTTG 840
 AAAATCCCAA AGTYAAAAGA AAAAAAAAAAN AAA 873

(2) INFORMATION FOR SEQ ID NO:75:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1238 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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- (A) LENGTH: 1432 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

GATGAAGAAG AGGAGGACAC CACCATCAAC AACTCCGACG TGGTGGTGCG CTACAAGAAG	60
GCCGCAACGT GGTGCAATGA AACGTTGCGC GTGCTTATCG ATGCCACAAA ACCTGGCGCC	120
AAGGTGTGCG ACCTGTGCCG CCTCGGTGAT GACACCATCA CCGCCNAGGT CAAGACAATG	180
TTCAAAGGCA CGGAAAAGG CATCGCTTTC CCGACCTGCA TCTCGGTCAA CAACTGCGTA	240
TGCCACAACA GCCCTGGCGT GTCGGACGAG ACGACGCAGC AAGAGATCGC GATGGGTGAC	300
GTCGTGCACT ACGACCTGGG CATCCACGTG GACGGCTACT GCGCCGTCGT CGCGCACACC	360
ATTCAGGTGA CAGAGGACAA TGAGCTTGGC AAGGACGAGA AGGCGGCGCG CGTCATTACA	420
GCGGCGTACA ACATCCTGAA CACGGCGCTG CGCCAGATGC GTCCCGGTAC GACCATCTAC	480
CAGGTGACAG ACGTAGTTGA GAAGGCTGCG GAGCACTACA AGGTGACTCC GGTAGACGGC	540
GTCCTCTCGC ATATGATGAA GCGCTACATC ATAGACNGAT ACCGCTGTAT CCCGCAGCGC	600
AGGGTCGCGG AGCACATGGT GCACGACTAC GATCTCGAGA AAGCGCAGGT GTGGACGCTA	660
GACATTGTCA TGACCTCCGG CAAGGGCAAG CTGAAGGAGC GCGATGCGCG GCCGTGCGTG	720
TTCAAGGTGG CTCTGGACTC CAACTACTCT GTGAAAATGG AAAGCGCGAA GGAGGTTGAG	780
AAGGAAATCG ACTCCNAGTA TGCCACCTTC CCCTTTGCCA TCCGCAACCT GGAGGCCAAG	840
AAGGCCCGCC TCGGTCTCAA CGAGATGGCG AAGCACGGTG CTGTCATCCC GTACCCTATT	900
CTCTTCGAAA AGGAAGGCGA GGTGCTCGCC CATTTCAAGA TTACGGTGCT CATCAGCAAC	960
AAGAAGATTG AGCCGATTAC CGGCCTGAAG CCGCAGAAGG CCCCAGCGCT CGAGCCATAC	1020
ACGGACGAGA TGCTGCTTGC GACGAACAAG CTCTTCGCTG TCGCTAGAGA AGAAGGCGGC	1080
GAAGTAGACG GCCGTGGCAT CCGTGACGCT GTACTGCGAG CTTTCGTAGG CGTACGCCTC	1140
TTGTGAGGCG TACACGTGTG CTGTTTGCGG ACGAGGAGGC ACCCATTTCTG TTCCCCTTCT	1200
TCGCTAATCT TCGCGTTTCC TCTGACGCTG GCTTCTYTGC CGGAGTGTGG TGAGGCGCGT	1260
GGGGGAGAAA CGGCCCACTY GCATGCCTGT GCATACGCGA GCACGGTAGG GAGCGCGGTG	1320

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

Gly Cys Gly Pro Lys Glu Asp Gly His Thr Gln Lys Asn Asp Gly Asp
 1 5 10 15

Gly Pro Lys Glu Asp Gly His Thr Gln Lys Asn Asp Gly Asp Gly Pro
 20 25 30

Lys Glu Asp Gly His Thr Gln Lys Asn Asp Gly Asp Gly
 35 40 45

(2) INFORMATION FOR SEQ ID NO:72:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 664 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

GCTGCAGGAA TTCGGCACGA GATTGCTTCC CAGCCCACCT TCGCTATCCA GCCACTCTCG	60
CTCTTCTACA TCTCCCACCC CCTCACACCG CCATGGCTTC TTCCCGCAAG GCTTCCAACC	120
CGCACAAGTC GCACCGCAAG CCGAAGCGCT CGTGGAAACGT GTACGTGGGC CGCTCGCTGA	180
AGGCGATCAA CGCCAGATG TCGATGTCGC ACCGCACGAT GAAGATCGTG AACTCGTACG	240
TGAACGACGT GATGGAGCGC ATCTGCACTG AGGCCGCGTC GATTGTTCGC GCGAACAAGA	300
AGCGCACGTT GGGTGCGCGC GAGGTGCAGA CGGCGGTGCG CATTGTGCTG CCGGCGGAGC	360
TCGCGAAGCA TGCCATGGCT GAGGGCACGA AGGCCGTGTC GAGCGCGTCC CGCTAAAGCG	420
GCTTGCCGGA TGCCGTGTGA GTAGGAGGGT GGCTTGCCGC AAACGCTGAC CTCGGCGATT	480
GCGGCGTGGC GCTCCCCTTC TCCTCCTTGT CCGGCGGTGT GTGTCATGCA TTTGCGTGAC	540
TCCTCCCTCT TATAGATGCA AGCTTTTTTT TTCTCTTGAC GTTTTATTTT CTCCTCCCCC	600
TCCCTTAACG TGAAGTGTAT ATGANAGCGT ACTGGACATG ANANAAAAAA AAAANAACT	660
CGAG	664

(2) INFORMATION FOR SEQ ID NO:73:

(i) SEQUENCE CHARACTERISTICS:

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Gly Pro Lys Glu Asp Gly Arg Thr Gln Lys Asn Asp Gly Asp Gly Pro
 20 25 30

Lys Glu Asp Gly Arg Thr Gln Lys Asn Asp Gly Asp Gly
 35 40 45

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 17 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

Gly Cys Gly Pro Lys Glu Asp Gly His Thr Gln Lys Asn Asp Gly Asp
 1 5 10 15

Gly

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 31 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

Gly Cys Gly Pro Lys Glu Asp Gly His Thr Gln Lys Asn Asp Gly Asp
 1 5 10 15

Gly Pro Lys Glu Asp Gly His Thr Gln Lys Asn Asp Gly Asp Gly
 20 25 30

(2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 45 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

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- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 17 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

Gly Cys Gly Pro Lys Glu Asp Gly Arg Thr Gln Lys Asn Asp Gly Asp
 1 5 10 15

Gly

(2) INFORMATION FOR SEQ ID NO:67:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 31 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

Gly Cys Gly Pro Lys Glu Asp Gly Arg Thr Gln Lys Asn Asp Gly Asp
 1 5 10 15

Gly Pro Lys Glu Asp Gly Arg Thr Gln Lys Asn Asp Gly Asp Gly
 20 25 30

(2) INFORMATION FOR SEQ ID NO:68:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 45 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

Gly Cys Gly Pro Lys Glu Asp Gly Arg Thr Gln Lys Asn Asp Gly Asp
 1 5 10 15

137

Pro Lys Glu Asp Gly Xaa Thr Gln Lys Asn Asp Xaa Xaa Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 6
- (D) OTHER INFORMATION: /note= "Xaa can be either His or

Arg"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

Pro Lys Glu Asp Gly Xaa Thr
1 5

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: /note= "Xaa can be either Gly or

Asp"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 6
- (D) OTHER INFORMATION: /note= "Xaa can be either Asp or

Gly"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

Gln Lys Asn Asp Xaa Xaa Gly
1 5

(2) INFORMATION FOR SEQ ID NO:66:

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Gly Asp Gly Pro Lys Glu Asp Gly Arg Thr Gln Lys Asn Asp Gly Asp
 130 135 140
 Gly Pro Lys Glu Asp Gly Arg Thr Gln Lys Asn Asp Gly Asp Gly Pro
 145 150 155 160
 Lys Glu Asp Gly Arg Thr Gln Lys Asn Asp Gly Asp Gly Pro Lys Glu
 165 170 175
 Asp Gly His Thr Gln Lys Asn Asp Gly Asp Gly Pro Lys Glu Asp Gly
 180 185 190
 Arg Thr Gln Lys Asn Asp Gly Gly Gly Pro Lys Glu Asp Glu Asn Leu
 195 200 205
 Gln Gln Asn Asp Gly Asn Ala Gln Glu Lys Asn Glu Asp Gly His Asn
 210 215 220
 Val Gly Asp Gly Ala Asn Gly Asn Glu Asp Gly Asn Asp Asp Gln Pro
 225 230 235 240
 Lys Glu Gln Val Ala Gly Asn
 245

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 6
- (D) OTHER INFORMATION: /note= "Xaa can be either His or Arg"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 12
- (D) OTHER INFORMATION: /note= "Xaa can be either Gly or Asp"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 13
- (D) OTHER INFORMATION: /note= "Xaa can be either Asp or Gly"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

135

Met Gly Ser Ser Cys Thr Lys Asp Ser Ala Lys Glu Pro Gln Lys Arg
 1 5 10 15

Ala Asp Asn Ile Asp Thr Thr Thr Arg Ser Asp Glu Lys Asp Gly Ile
 20 25 30

His Val Gln Glu Ser Ala Gly Pro Val Gln Glu Asn Phe Gly Asp Ala
 35 40 45

Gln Glu Lys Asn Glu Asp Gly His Asn Val Gly Asp Gly Ala Asn Asp
 50 55 60

Asn Glu Asp Gly Asn Asp Asp Gln Pro Lys Glu Gln Val Ala Gly Asn
 65 70 75 80

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 247 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

Met Gly Ala Tyr Cys Thr Lys Asp Ser Ala Lys Glu Pro Gln Lys Arg
 1 5 10 15

Ala Asp Asn Ile His Lys Thr Thr Glu Ala Asn His Arg Gly Ala Ala
 20 25 30

Gly Val Pro Pro Lys His Ala Gly Gly Ala Met Asn Asp Ser Ala Pro
 35 40 45

Lys Glu Asp Gly His Thr Gln Lys Asn Asp Gly Asp Gly Pro Lys Glu
 50 55 60

Asp Gly Arg Thr Gln Lys Asn Asp Asp Gly Gly Pro Lys Glu Asp Gly
 65 70 75 80

His Thr Gln Lys Asn Asp Gly Asp Gly Pro Lys Glu Asp Gly Arg Thr
 85 90 95

Gln Lys Asn Asn Gly Asp Gly Pro Lys Glu Asp Gly His Thr Gln Lys
 100 105 110

Asn Asp Gly Asp Ala Pro Lys Glu Asp Gly Arg Thr Gln Lys Asn Asp
 115 120 125

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AAGGAGCAGG TTGCCGGCAA CTAG

264

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 744 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

ATGGGAGCCT ACTGCACGAA GGACTCCGCA AAGGAGCCCC AGAAGCGTGC TGATAACATC	60
CATAAAACCA CTGAGGCCAA TCACAGAGGC GCCGCCGGTG TGCCCCCGAA GCACGCCGGC	120
GGTGCGATGA ACGACTCTGC CCCGAAGGAG GATGGCCATA CACAGAAAAA TGACGGCGAT	180
GGCCCTAAGG AGGACGGCCG TACACAGAAA AACGACGACG GTGGCCCTAA GGAGGACGGC	240
CATACACAGA AAAATGACGG CGATGGCCCT AAGGAGGACG GCCGTACACA GAAAAATAAC	300
GGCGATGGCC CTAAGGAGGA CGGCCATACA CAGAAAAATG ACGGCGATGC CCCTAAGGAG	360
GACGGCCGTA CACAGAAAAA TGACGGCGAT GGCCCTAAGG AGGACGGCCG TACACAGAAA	420
AATGACGGCG ATGGCCCTAA GGAGGACGGC CGTACACAGA AAAATGACGG CGATGGCCCT	480
AAGGAGGACG GCCGTACACA GAAAAATGAC GGCGATGGCC CTAAGGAGGA CGGCCATACA	540
CAGAAAAATG ACGGCGATGG CCCTAAGGAG GACGGCCGTA CACAGAAAAA TGACGGCGGT	600
GGCCCTAAGG AGGATGAGAA TCTGCAGCAA AACGATGGGA ATGCGCAGGA GAAGAACGAA	660
GATGGACACA ACGTGGGGGA TGGAGCTAAC GGCAATGAGG ATGGTAACGA TGATCAGCCG	720
AAGGAGCAGG TTGCCGGCAA CTAG	744

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 80 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

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(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

Pro Lys Glu Asp Gly His Ala
1 5

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

Pro Lys Asn Asp Asp His Ala
1 5

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 264 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

ATGCACCATC ATCACCATCA CATGGGAAGC TCCTGCACGA AGGACTCCGC AAAGGAGCCC	60
CAGAAGCGTG CTGATAACAT CGATACGACC ACTCGAAGCG ATGAGAAGGA CGGCATCCAT	120
GTCCAGGAGA GCGCCGGTCC TGTGCAGGAG AACTTCGGGG ATGCGCAGGA GAAGAACGAA	180
GATGGACACA ACGTGGGGGA TGGAGCTAAC GACAATGAGG ATGGTAACGA TGATCAGCCG	240

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Arg Leu Ser Asp Ala Cys Ser Val Pro Asn Cys Lys Lys Cys Glu Thr
 180 185 190
 Gly Thr Ser Arg Leu Cys Ala Glu Cys Asp Thr Gly Tyr Ser Leu Ser
 195 200 205
 Ala Asp Ala Thr Ser Cys Ser Ser Pro Thr Thr Gln Pro Cys Glu Val
 210 215 220
 Glu His Cys Asn Thr Cys Val Asn Gly Asp Ser Thr Arg Cys Ala Tyr
 225 230 235 240
 Cys Asn Thr Gly Tyr Tyr Val Ser Asp Gly Lys Cys Lys Ala Met Gln
 245 250 255
 Gly Cys Tyr Val Ser Asn Cys Ala Gln Cys Met Leu Leu Asp Ser Thr
 260 265 270
 Lys Cys Ser Thr Cys Val Lys Gly Tyr Leu Leu Thr Ser Ser Tyr Ser
 275 280 285
 Cys Val Ser Gln Lys Val Ile Asn Ser Ala Ala Ala Pro Tyr Ser Leu
 290 295 300
 Trp Val Ala Ala Ala Val Leu Leu Thr Ser Phe Ala Met His Leu Ala
 305 310 315 320

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

Pro Lys Glu Asp Gly His Ala Pro Lys Asn Asp Asp His Ala
 1 5 10

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

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CAGCCACTCC AACCACGAAA GCTCTCTTCT GCGCACATAA AAAAAAAAAA AAAAAAAAAA 1560

CTCGAGGGGG GGCCCGGTAC CCAA 1585

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 320 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

Val Leu Pro Asp Met Thr Cys Ser Leu Thr Gly Leu Gln Cys Thr Asp
1 5 10 15

Pro Asn Cys Lys Thr Cys Thr Thr Tyr Gly Gln Cys Thr Asp Cys Asn
20 25 30

Asp Gly Tyr Gly Leu Thr Ser Ser Ser Val Cys Val Arg Cys Ser Val
35 40 45

Ala Gly Cys Lys Ser Cys Pro Val Asp Ala Asn Val Cys Lys Val Cys
50 55 60

Leu Gly Gly Ser Glu Pro Ile Asn Asn Met Cys Pro Cys Thr Asp Pro
65 70 75 80

Asn Cys Ala Ser Cys Pro Ser Asp Ala Gly Thr Cys Thr Gln Cys Ala
85 90 95

Asn Gly Tyr Gly Leu Val Asp Gly Ala Cys Val Arg Cys Gln Glu Pro
100 105 110

Asn Cys Phe Ser Cys Asp Ser Asp Ala Asn Lys Cys Thr Gln Cys Ala
115 120 125

Pro Asn Tyr Tyr Leu Thr Pro Leu Leu Thr Cys Ser Pro Val Ala Cys
130 135 140

Asn Ile Glu His Cys Met Gln Cys Asp Pro Gln Thr Pro Ser Arg Cys
145 150 155 160

Gln Glu Cys Val Ser Pro Tyr Val Val Asp Ser Tyr Asp Gly Leu Cys
165 170 175

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

AAAGCTGGAG CTCCACCGCG GTGGCGGCCG CTCTAGAACT AGTGGATCCC CCGGGCTGCA	60
GGAATTCGGC ACGAGTGCTG CCCGACATGA CATGCTCGCT GACCGGACTT CAGTGCACAG	120
ACCCGAACTG CAAGACCTGC ACAACTTACG GTCAGTGCAC AGACTGCAAC GACGGCTACG	180
GTCTCACCTC CTCCAGCGTT TGCCTGCGCT GCAGTGTAGC GGGCTGCAAG AGCTGCCCCG	240
TCGACGCTAA CGTCTGCAAA GTGTGTCTCG GCGGCAGCGA GCCGATCAAC AATATGTGCC	300
CCTGCACCGA CCCCAACTGC GCCAGCTGCC CCAGCGACGC TGGCACGTGC ACTCAGTGCG	360
CGAACGGCTA CGGTCTCGTG GACGGCGCCT GTGTGAGATG CCAGGAGCCC AACTGCTTCA	420
GCTGCGACAG CGACGCGAAT AAGTGCACAC AATGTGCGCC GAACTACTAC CTCACCCCGC	480
TCTTGACCTG CTCCCCGGTG GCCTGCAACA TCGAGCACTG CATGCAGTGC GACCCACAGA	540
CGCCGTCGCG CTGCCAGGAG TGCCTGTCCC CCTACGTGGT TGACAGCTAC GACGGCCTCT	600
GCAGGCTCTC CGATGCCTGC TCCGTGCCCA ACTGCAAGAA GTGCGAGACC GGTACCTCCA	660
GGCTCTGCGC CGAGTGCGAC ACCGGCTACA GTCTCTCCGC CGACGCGACG AGCTGCAGCA	720
GTCCAACCAC GCAGCCGTGC GAGGTGGAGC ACTGCAACAC ATGTGTGAAC GGCGATAGCA	780
CCCGCTGTGC CTA CTGCAAC ACCGGCTACT ACGTCTCCGA TGGCAAGTGC AAGGCCATGC	840
AGGGCTGCTA CGTGTCGAAC TGCGCGCAGT GCATGCTGCT TGACAGCACC AAGTGCTCCA	900
CGTGCGTGAA AGGGTACCTG CTCACGTCGT CCTACAGTTG CGTCTCGCAG AAAGTCATCA	960
ACAGTGCGGC CGCGCCCTAC TCTCTGTGGG TGGCCGCCGC CGTGCTCCTC ACCTCTTTTG	1020
CCATGCACCT AGCATAGTGC GCAGCGGCAT GCGAACAACC CCACTCTCAT TCTCCAACAT	1080
GTGCATACAC ACACACACAG ACAGCGGGGC AGCACCCCT CCCCACACAC ACACACGCAC	1140
TTCCCCCTTG TCTTGTTCTT CTTTCCTCGN TTCGCATTTT TTTCTCTCGT GCGCTGGCGC	1200
CGGCCTCCTG CACGTCGCTC CCCTCCCCCT AACCTCTATT CTCTCTCTCT CTCTCTCTCG	1260
CCGGCATCAT TGCTTCTTAC CCTTTTCTGA TCCTTGCTCG CGTGGGCGGA CACTGCCACA	1320
GTCCCACAGC GCAGACACAC GTGTTTAAAC GCGCGAGGCA TCCCTCCCTA TCACTTCATT	1380
TCTCCTAAAG CCACTCACCA AGTCGCACAC CGCCCTCCCC CATCGGCCGC CCTTCCGGGC	1440
GCAGCTGTGC GGAATGGGTG TGTGCTCGAC CTCGTTCTTG GCAGCTCACT CGCATGTGTA	1500

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Asp Ile Arg Ala Arg Lys Gly Ser Val Pro Cys Gly Ala Ser Ser Met
 100 105 110
 Glu Asn Ser Thr Pro Leu Asp Ser Ala Val Glu Pro Phe Glu Ser Asp
 115 120 125
 Asp Gly Asp Asp Val Val Asp Lys Thr Gly Leu Asp Pro Asn Glu Leu
 130 135 140
 Gln Gly Ile Ile Pro Arg Ala Cys Thr Asp Leu Phe Asp Gly Leu Arg
 145 150 155 160
 Ala Lys Arg Ala Lys Asp Ser Asp Phe Thr Tyr Arg Val Glu Val Ser
 165 170 175
 Tyr Tyr Glu Ile Tyr Asn Glu Lys Val Phe Asp Leu Ile Arg Pro Gln
 180 185 190
 Arg Asn Thr Asp Leu Arg Ile Arg Asn Ser Pro Asn Ser Gly Pro Phe
 195 200 205
 Ile Glu Gly Leu Thr Trp Lys Met Val Ser Lys Glu Glu Asp Val Ala
 210 215 220
 Arg Val Ile Arg Lys Gly Met Gln Glu Arg His Thr Ala Ala Thr Lys
 225 230 235 240
 Phe Asn Asp Arg Ser Ser Arg Ser His Ala Ile Leu Thr Phe Asn Ile
 245 250 255
 Val Gln Leu Ser Met Asp Asp Ser Asp Asn Ala Phe Gln Met Arg Ser
 260 265 270
 Lys Leu Asn Leu Val Asp Leu Ala Gly Ser Glu Arg Thr Gly Ala Ala
 275 280 285
 Gly Ala Glu Gly Asn Glu Phe His Asp Gly Val Lys Ile Asn His Ser
 290 295 300
 Leu Thr Val Leu Gly Arg Val Ile Asp Arg Leu Ala Asp Leu Ser Gln
 305 310 315 320
 Asn Lys Gly Gly

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1585 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

128

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Leishmania chagasi*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

Val Arg Glu Thr Arg Thr Cys Met Leu Leu Val Glu Gly Ala Glu Leu
 1 5 10 15

Val Pro Leu Met His Lys His Glu Val Val His Cys Leu Val Arg His
 20 25 30

Phe Pro Glu His Asn Glu Gln Arg His His Thr Ala Ser Asn Ser Arg
 35 40 45

Gln Ser Glu Cys Asp Ala His Thr Lys Ser Lys Val Val Asp Gln
 50 55 60

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 324 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Leishmania chagasi*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

Phe Arg Gly Ile Pro Arg Asn Ser Val Arg Arg Gly Thr Arg Thr Gly
 1 5 10 15

Val Asp Ser Asp Ala Thr Ser Ser Thr Thr Ala Ala Tyr Asp Gly Ala
 20 25 30

Gly Ser Ala Pro Val Met Val Asp Ala Asn Val Ser His Pro Pro Tyr
 35 40 45

Ala Gly His Asp Gln Val Tyr Met His Val Gly Lys Pro Ile Val Gly
 50 55 60

Asn Thr Leu Asp Gly Tyr Asn Gly Cys Val Phe Ala Tyr Gly Xaa Thr
 65 70 75 80

Gly Ser Gly Lys Thr Phe Thr Met Leu Gly Tyr Ala Pro Ser Thr Xaa
 85 90 95

127

465 470 475 480

Ala Cys Ala Pro Val Ser Ser Ala Pro Ser Trp Lys Ser Ser Arg Arg
 485 490 495

Arg Ser Gly Ile Met Gly Ala Cys Thr Arg Arg Thr Thr Ser
 500 505 510

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 107 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Leishmania chagasi*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

Gly Arg Arg Met Ala Ile His Arg Lys Met Thr Ala Met Ala Leu Arg
 1 5 10 15

Arg Thr Ala Val His Arg Lys Thr Thr Thr Val Ala Leu Arg Arg Thr
 20 25 30

Ala Ile His Arg Lys Met Thr Ala Met Ala Leu Arg Arg Thr Ala Val
 35 40 45

His Arg Lys Ile Thr Ala Met Ala Leu Arg Arg Thr Ala Ile His Arg
 50 55 60

Lys Met Thr Ala Met Pro Leu Arg Arg Thr Ala Val His Xaa Lys Met
 65 70 75 80

Thr Ala Met Ala Leu Arg Arg Thr Ala Val His Arg Lys Met Thr Ala
 85 90 95

Met Ala Leu Arg Xaa Thr Pro Tyr Thr Glu Lys
 100 105

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 63 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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Gly Ser Tyr Leu Ala Ala Gly Thr Ser Leu Ser Pro Tyr Glu Ala Leu
 180 185 190
 Met Asn Glu Tyr Glu Pro Gly Ile Thr Thr Gln Lys Leu Asp Glu Val
 195 200 205
 Tyr Ala Asn Val Lys Ser Trp Leu Pro Gln Leu Leu Lys Asp Ile Val
 210 215 220
 Gln Lys Gln Ser Gly Glu Ser Val Ile Ala Phe Ser His Lys Phe Pro
 225 230 235 240
 Gln Asp Lys Gln Glu Ala Leu Cys Lys Glu Phe Met Lys Ile Trp His
 245 250 255
 Phe Asp Thr Asp Ala Gly Arg Leu Asp Val Ser Pro His Pro Phe Thr
 260 265 270
 Gly Met Thr Lys Glu Asp Cys Arg Leu Thr Thr Asn Tyr Ile Glu Asp
 275 280 285
 Thr Phe Val Gln Ser Leu Tyr Gly Val Ile His Glu Ser Gly His Gly
 290 295 300
 Lys Tyr Glu Gln Asn Cys Gly Pro Arg Glu His Ile Thr Gln Pro Val
 305 310 315 320
 Cys Asn Ala Arg Ser Leu Gly Leu His Glu Ser Gln Ser Leu Phe Ala
 325 330 335
 Glu Phe Gln Ile Gly His Ala Thr Pro Phe Ile Asp Tyr Leu Thr Thr
 340 345 350
 Arg Leu Pro Glu Phe Phe Glu Ala Gln Pro Ala Phe Ser Gln Asp Asn
 355 360 365
 Met Arg Lys Ser Leu Gln Gln Val Lys Pro Gly Tyr Ile Arg Val Asp
 370 375 380
 Ala Asp Glu Val Cys Tyr Pro Leu His Val Ile Leu Arg Tyr Glu Ile
 385 390 395 400
 Glu Arg Asp Leu Met Glu Gly Lys Met Glu Val Glu Asp Val Pro Arg
 405 410 415
 Ala Trp Asn Ala Lys Met Gln Glu Tyr Leu Gly Leu Ser Thr Glu Gly
 420 425 430
 Arg Asp Asp Val Gly Cys Leu Gln Asp Val His Trp Ser Met Val Arg
 435 440 445
 Ser Ala Thr Leu Arg Arg Thr Arg Ser Ala Pro Cys Met Arg Arg Arg
 450 455 460
 Ser Trp Arg Ala Ser Glu Arg Ser Trp Glu Thr Thr Arg Trp Met Ser

125

115

120

125

Arg Lys Thr Gly Lys Ala Gly Thr
130 135

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 510 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Leishmania chagasi*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

Tyr Leu Leu Pro Leu Leu Gly Arg Arg Thr Thr Thr Thr Phe Lys Thr
1 5 10 15

Thr Pro Arg Leu Leu Val Pro His Leu Leu Leu Ser Thr Phe Asn Pro
20 25 30

Cys Leu Ala Asn Met Glu Ala Tyr Lys Lys Leu Glu Thr Ile Phe Thr
35 40 45

Lys Val Tyr Arg Leu Asp His Phe Leu Gly Leu Gly Asn Trp Asp Met
50 55 60

Asn Thr Asn Met Pro Pro Lys Gly Glu Glu Ser Arg Gly Glu Ala Met
65 70 75 80

Ala Met Leu Ser Glu Leu Arg Phe Gly Phe Ile Thr Ala Pro Glu Val
85 90 95

Lys Ser Leu Ile Glu Ser Ala Thr Lys Gly Ser Glu Glu Leu Asn Ala
100 105 110

Val Gln Arg Ala Asn Leu Arg Glu Met Arg Arg Ala Trp Lys Ser Ala
115 120 125

Thr Ala Leu Pro Ala Glu Phe Val Gly Arg Lys Met Arg Leu Thr Thr
130 135 140

His Ala His Ser Val Trp Arg Asp Ser Arg Lys Ala Asn Asp Phe Ala
145 150 155 160

Lys Phe Leu Pro Val Leu Arg Asp Leu Val Ala Leu Ala Arg Glu Glu
165 170 175

124

GGTGTCTTAC TACGAGATCT ACAACGAGAA GGTGTTTCGAT CTCATCCGGC CGCAGCGCAA 660
CACGGACCTG AGGATACGTA ACTCGCCCAA CTCCGGTCCA TTTATCGAAG GCCTGACGTG 720
GAAGATGGTG TCCAAGGAGG AAGACGTCGC CCGCGTGATT CGCAAGGGCA TGCAGGAGCG 780
CCACACGGCT GCGACCAAGT TCAACGACCG CAGCAGCCGC AGCCACGCCA TCCTCACCTT 840
CAACATTGTG CAGCTGTCGA TGGACGACTC CGACAACGCG TTCCAGATGC GCAGCAAGCT 900
GAACCTGGTG GACCTTGCTG GGTCCGAGCG CACTGGTGCG GCCGGAGCCG AGGGCAATGA 960
GTTCCACGAC GGTGTGAAGA TCAACCACTC GCTGACGGTG CTGGGGCGCG TGATCGACCG 1020
TCTGGCGGAC CTCTCGCAGA ACAAGGGAGG GGG 1053

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 136 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Leishmania chagasi*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

Gly Arg Leu Ser Glu Glu Glu Ile Glu Arg Met Val Arg Glu Ala Ala
1 5 10 15
Glu Phe Glu Asp Glu Asp Arg Lys Val Arg Glu Arg Val Glu Ala Lys
20 25 30
Asn Ser Leu Glu Ser Ile Ala Tyr Ser Leu Arg Asn Gln Ile Asn Asp
35 40 45
Lys Asp Lys Leu Gly Asp Lys Leu Ala Ala Asp Asp Lys Lys Ala Ile
50 55 60
Glu Glu Ala Val Lys Asp Ala Leu Asp Phe Val His Glu Asn Pro Asn
65 70 75 80
Ala Asp Arg Glu Glu Phe Glu Ala Ala Arg Thr Lys Leu Gln Ser Val
85 90 95
Thr Asn Pro Ile Ile Gln Lys Val Tyr Gln Gly Ala Ala Gly Ser Gly
100 105 110
Ala Glu Glu Ala Asp Ala Met Asp Asp Leu Leu Val Gly Arg Val Lys

TCATCATACA GCATCCAATA GCCGCCAGAG TGAATGCGAT GCGCACACCA AGTCGAAAGT	180
GGTCGACCAG TAGGGGAATG TGACCCTGGC TGGCGTGCAA CATGATCGCC ACGCCAGCGG	240
TGGGCCACAC CACAACAGAG GCGACGAAAG AGAACATGAA CTTGCTCACG AAGCTNACAA	300
TAAGGGCGTC GCTNGTGATG CTAAGAACCA CGCCNAGGTA GACGGCGAAG ANCAAATAA	360
ACACAAGCGT GACGATCCCG AAAAGAAGGA TCTCTGCGGA ATTTTCGTGA GATAGANAAT	420
GCCCGTACTG GAAAAANAAG CCGGCAGGCG CGCGATAACG CTGCAACTTG CCGCTCCTCG	480
CGGGCGCGTT TTCGCTCCTT CTCCGACTTG ATGGCGCNGT CNGNCTTGAC AAAACGGTTA	540
AGCTCCTCAT GCCCCAGCCG ATTCCCAGCT CACGGTCCAC TTCCGGCCAT GCCCACGGAC	600

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1053 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Leishmania chagasi*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

GGGAAAAAAG TGGAGCTCCA CCGCGGTGGC GGCCGCTCTA GAACTAGTGG ATCCCCCGGG	60
CTGCAGGAAT TCCGCGGAAT TCCGCGGAAT TCCGCGGAAT TCCGTCCGAC GCGGCACCCG	120
CACAGGGGTC GACAGTGACG CAACCTCCTC CACCACTGCG GCCTACGACG GCGCCGGCTC	180
CGCGCCAGTG ATGTTGACG CCAATGTGAG CCACCCTCCG TACGCGGGGC ATGACCAAGT	240
GTACATGCAC GTCGGCAAGC CCATCGTGGG CAACACCCTC GACGGATACA ACGGGTGCGT	300
GTTGCGCTAC GGGCANACGG GCAGCGGCAA AACCTTCACG ATGCTCGGNT ACGCGCCGAG	360
CACGANCGAC ATCCGCGCTC GCAAAGGGTC CGTCCCCTGC GGGGCCAGCA GCATGGAGAA	420
CAGCACTCCT CTTGACAGCG CTGTGGAGCC GTTTGAGAGC GATGACGGCG ACGACGTGGT	480
GGACAAGACG GGGCTGGATC CGAACGAGCT GCAAGGCATC ATCCCGCGCG CGTGACGGA	540
CCTGTTTCGAT GGTCTCCGTG CGAAGCGCGC CAAGGACTCC GACTTCACGT ACCGCGTGGA	600

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(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 560 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Leishmania chagasi*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

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CGGAAGGAGG ATGCCATAC ACAGAAAAAT GACGGCGATG GCCCTAAGGA GGACGGCCGT      60
ACACAGAAAA ACGACGACGG TGGCCCTAAG GAGGACGGCC ATACACAGAA AAATGACGGC      120
GATGGCCCTA AGGAGGACGG CCGTACACAG AAAAATAACG GCGATGGCCC TNAGGAGGAC      180
GGCCATACAC AGAAAAATGA CGGCGATGCC CCTNAGGAGG ACGGCCGTAC ACANAAAAAT      240
GACGGCNATG GCCCTNAGGA GGACGGCCGT ACACAGAAAA ATGACNGCCA TGGCCCTTAG      300
GANGACGCCG TACACAGAAA AATGACGCNA TGGCCCTNAG GGAGGACGGC CATACCCANA      360
AAAATTGACG GCNATNGCCC TTAGGANGAC GGCCGTNCCC ANAAANANTG ACNGCGGTNG      420
CCCTTAAGGA AGATGAAAAT CTGCCACCAA AACNATTGGG AATGCNCAGG AAAANAACNA      480
ANATNGACCC CACGTGGGGG ATGGANCTTA CNGCNATTAA NATTGTTACC ATTATCNACC      540
NAAGGACNNG TTGCCGNCAA
                                                                                   560

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(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 600 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Leishmania chagasi*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

```

CGTCCGAGAA ACCCGTACAT GTATGCTGCT GGTAGAAGGC GCAGAGCTGG TCCCTCTGAT      60
GCACAAGCAT GAGGTCGTAC ATTGCCTGGT TCGTCATTTT CCAGAGCACA ACGAGCAGCG      120

```

ATGCCCCCA AGGGCGAGGA ATCACGCGGT GAGGCGATGG CGATGCTCTC GGAGCTCCGC	300
TTTGGCTTCA TCACGGCACC GGAGGTGAAA AGCCTGATTG AGAGTGCCAC CAAGGGCAGC	360
GAGGAGCTGA ATGCGGTGCA GCGCGCTAAC TTGCGGGAGA TGAGGCGTGC GTGGAAGAGC	420
GCCACCGCCT TGCCGGCTGA GTTTGTGGGC CGCAAGATGC GCCTCACGAC ACACGCGCAC	480
AGCGTGTGGC GCGACAGCCG CAAAGCAAAT GACTTCGCCA AGTTCCTACC GGTGCTCAGG	540
GACCTGGTGG CGCTCGCCCG TGAGGAGGGC TCATACCTCG CCGCCGGCAC CTCCCTCTCC	600
CCGTATGAGG CGCTCATGAA CGAGTACGAG CCAGGAATCA CGACACAAAA GCTGGATGAG	660
GTGTACGCAA ATGTAAAGTC GTGGCTGCCG CAGCTGCTAA AGGACATTGT GCAGAAGCAG	720
TCCGGCGAGT CGGTGATTGC GTTCTCGCAT AAGTTCCCGC AGGACAAGCA GGAAGCACTG	780
TGCAAGGAAT TCATGAAGAT CTGGCACTTC GACACCGATG CCGGTGCGCT CGACGTCAGC	840
CCCCACCCTT TCACGGGAAT GACGAAGGAG GACTGCCGAC TCACAACAAA CTACATCGAA	900
GACACGTTTG TTCAGAGCTT GTATGGCGTC ATCCACGAGA GTGGGCATGG CAAGTACGAG	960
CAGAACTGTG GCCCACGCGA GCACATCACG CAGCCGGTGT GCAACGCCCC CTCTCTTGGC	1020
CTGCATGAGA GCCAGAGCCT CTTTGCGGAG TTTCAGATCG GCCACGCGAC GCCCTTCATC	1080
GACTACCTCA CAACTCGCCT TCCTGAGTTC TTCGAGGCGC AGCCAGCGTT CTCGCAGGAC	1140
AACATGCGCA AGTCGCTGCA GCAGGTGAAG CCGGGCTACA TTCGCGTCGA TGCCGATGAG	1200
GTGTGCTACC CTCTGCACGT GATCCTGCGC TACGAGATCG AGCGCGACTT GATGGAGGGC	1260
AAAATGGAGG TGGAAGACGT GCCGCGCGCG TGGAACGCAA AGATGCAGGA GTACTTGGGT	1320
CTCTCAACGG AGGGCCGTGA CGACGTTGGG TGCCTGCAGG ACGTGCATTG GTCCATGGTG	1380
CGCTCGGCTA CTCTCCGACG TACTCGCTCG GCGCCATGTA TGCGGCGCAG ATCATGGCGA	1440
GCATCCGAAA GGAGCTGGGA GACGACAAGG TGGATGAGTG CCTGCGCACC GGTGAGCTCG	1500
GCCCCCTCCT GGAAAAGCAG CAGGAGAAGA TCTGGGATCA TGGGTGCCTG TACGAGACGG	1560
ACGACCTCAT GACGCGTGCG ACGGGCGAGA CGCTGAACCC CGAGTACCTG CGCCGCCACC	1620
TGGAGGCGCG CTACATAAAC GCCTGAGTCG CGAGCGGTTG ACACACGCGC TCGCTAGCAC	1680
ATGACGCGTC TTTATTATTC TTTGTTGTGC ATTCGGAATT CCGCGGAATT CGATATCAAG	1740
CTTATCGA	1748

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(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Leishmania chagasi*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

CGGCCGCTC AGCGAGGAGG AGATCGAGCG CATGGTGCGC GAGGCTGCCG AGTTCGAGGA	60
TGAGGACCGC AAGGTGCGCG AACGTGTCGA AGCGAAGAAC TCGCTAGAGA GCATCGCGTA	120
CTCGCTTCGC AACCAGATCA ACGACAAGGA CAAGCTTGGT GACAAGCTCG CCGCGGACGA	180
CAAGAAGGCG ATCGAGGAGG CTGTGAAGGA TGCCCTCGAC TTTGTCCACG AGAACCCCAA	240
TGCAGACCGT GAGGAGTTCG AGGCTGCTCG CACGAAGCTG CAGAGTGTGA CGAACCCCAT	300
CATTCAAAAG GTGTACCAGG GCGCCGCCGG CTCTGGTGCA GAAGAGGCGG ACGCGATGGA	360
TGACTTGTTA GTCGGCCGCG TGAAAAGAAA AACAGGGAAA GCGGGAACAT NCCACAANAA	420
CCNAAGAAGA AAGGGGGTNG CGACACCGCT CGAACACCGA CGGCNCACAT NCNTCATGGG	480
CATGCTCAGC TTTCTCTCTC CCAACAAACC AGAAGGTTTT CTCCAAACNC CGTCTCNGCN	540
CCCAAAATAC GGAAANGTTA ANCGAAAAAN CCCCTTCCAC CAATTGNNGT TCTTTTGTTT	600

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1748 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Leishmania chagasi*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

CTAGTGGATC CCCCGGGCTG CAGGAATTCA CGGAATACGT ACCTCCTCCC CTTTCTTGGT	60
AGAAGAACAA CAACAACGTT CAAGACGACG CCGCGCCTTC TTGTACCGCA TTTGCTTCTG	120
AGCACGTTCA ATCCGTGCCT TGCAAACATG GAGGCGTACA AGAAGCTGGA AACGATCTTT	180
ACGAAGGTCT ACCGCCTGGA CCACTTCCTC GGTCTGGGCA ACTGGGACAT GAACACAAAC	240

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Ala Arg Xaa Ile Phe Val Lys Thr Leu Thr Gly Xaa Thr Ile Ala Leu
 1 5 10 15

Glu Val Glu Pro Ser Asp Thr Ile Glu Asn Val Lys Ala Lys Ile Gln
 20 25 30

Asp Lys Glu Gly Ile Pro Pro Asp Gln Gln Arg Leu Ile Phe Ala Gly
 35 40 45

Lys Gln Leu Glu Xaa Gly Arg Thr Leu Ser Asp Tyr Asn Ile Gln Lys
 50 55 60

Glu Ser Thr Leu His Leu Val Leu Arg Leu Arg Gly Gly Met Xaa Ile
 65 70 75 80

Phe Val Lys Thr Leu Thr Gly Xaa Thr Ile Ala Leu Glu Val Glu Pro
 85 90 95

Asn Asp

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 39 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Leishmania major

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Leu Gln Gln Arg Leu Asp Thr Ala Thr Gln Gln Arg Ala Glu Leu Glu
 1 5 10 15

Ala Arg Val Ala Arg Leu Ala Ala Asp Arg Asp Glu Ala Arg Gln Gln
 20 25 30

Leu Ala Ala Asn Ala Glu Glu
 35

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 600 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

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(A) ORGANISM: *Leishmania major*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

```

Leu Gln Gln Arg Leu Asp Thr Ala Thr Gln Gln Arg Ala Glu Leu Glu
1           5           10           15
Ala Arg Val Ala Arg Leu Ala Ala Asp Arg Asp Glu Ala Arg Gln Gln
          20           25           30
Leu Ala Ala Asn Ala Glu Glu Leu Gln Gln Arg Leu Asp Thr Ala Thr
          35           40           45
Gln Gln Arg Ala Glu Leu Glu Ala Arg Val Ala Arg Leu Ala Ala Asp
          50           55           60
Gly Asp Glu Ala Arg Gln Gln Leu Ala Ala Asn Ala Glu Glu Leu Gln
          65           70           75           80
Gln Arg Leu Asp Thr Ala Thr Gln Gln Arg Ala Glu Leu Glu Ala Gln
          85           90           95
Val Ala Arg Leu Ala Ala Asn Ala Glu Glu Leu Gln Gln Arg Leu Asp
          100          105          110
Thr Ala Thr Gln Gln Arg Ala Glu Leu Glu Ala Arg Val Ala Arg Leu
          115          120          125
Ala Ala Asp Arg Asp Glu Ala Arg Gln Gln Leu Ala Ala Asn Ala Glu
          130          135          140
Glu Leu Gln Gln Arg Leu Asp Thr Ala Thr Gln Gln Arg Ala Glu Leu
          145          150          155          160
Glu Ala Gln Val Ala Arg Leu Ala Ala
          165

```

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 98 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Leishmania major*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

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Gln Gly Gln Xaa Phe Val Gly Asn Ser Leu Val Val Arg Leu Ile Asp
 20 25 30

Xaa Leu Xaa Gln Xaa Pro Ala Gly Tyr Pro Val Tyr Xaa Asn Arg Gly
 35 40 45

Ala Xaa Gly Ile Xaa Xaa Leu Leu Ser Thr Ala Ala Gly Val Xaa Arg
 50 55 60

Ala
 65

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 78 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Leishmania major*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Asp Asp His Ala Pro Lys Glu Asp Gly His Ala Pro Lys Asn Asp Asp
 1 5 10 15

His Ala Pro Lys Glu Asp Gly His Ala Pro Lys Asn Asp Asp His Ala
 20 25 30

Pro Lys Glu Asp Gly His Ala Pro Lys Asn Asp Gly Asp Val Gln Xaa
 35 40 45

Lys Ser Glu Asp Gly Asp Asn Val Gly Glu Gly Gly Lys Gly Asn Glu
 50 55 60

Asp Gly Asn Asp Asp Gln Pro Lys Glu His Ala Ala Gly Asn
 65 70 75

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 169 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:

116

Asn Asn Ala Arg Gln Ala Glu Asp Pro Ala Arg Leu Ile
 65 70 75

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 68 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Leishmania major*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

His Glu Pro Gln Trp Ser Ser Met Lys Ile Leu Gln Tyr Leu Thr Leu
 1 5 10 15

Asp Gly Thr Gln Val Ser Gly Thr Leu Pro Pro Gln Trp Ser Ala Met
 20 25 30

Ala Ser Val Arg Ile Leu Asn Leu Xaa Gly Thr Glu Val Ser Gly Thr
 35 40 45

Leu Pro Pro Glu Trp Ile Ser Met Xaa Arg Leu Gln Thr Leu Asn Leu
 50 55 60

Arg Arg Thr Lys
 65

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 65 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Leishmania major*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Ala Arg Glu Ala Gln Leu Ala His Arg Ile Cys Asp Tyr Leu Pro Glu
 1 5 10 15

115

	20		25		30
Leu Val Val Ala His Glu Gly Asp Gly Gln Ser Ser Ala Ala His Ala	35		40		45
Ala Ser Ala Ala Asp Ala Val Asn Val Leu Leu Thr Arg Glu Arg Gln	50		55		60
Ile Val Ala Glu Asp Glu Arg Asp Ala Leu His Ile Asn Ala Thr Arg	65		70		75
					80
Pro Gln Val Arg Cys Asn Xaa His Ala Ala Val Ser Ile Thr Glu Cys		85		90	95
Arg Ile Ile Ser Ser Arg Ser Ala Trp Gly Met Ser Pro Cys Met Ala		100		105	110
Asp Thr Ala Lys Phe Ser Arg Val Ile Phe Leu Ala Ser Cys Ser Thr		115		120	125
Phe Leu Arg Val Leu Xaa Lys Thr Thr Ala Trp Ala Ile Val Lys Leu		130		135	140
Ser Tyr					
145					

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 77 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Leishmania major

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Ala Gln Gly Phe Arg Val Ile Phe Thr His Gly Gly Ala Gln Met Gly			
1	5	10	15
Glu Val Asn Thr Arg Thr Glu Leu Leu Asp His Ile Asn Gln Ile Val			
	20	25	30
Val Ser Thr His Ala Val Xaa Thr Gly Ala His Gly Lys Thr Val Cys			
	35	40	45
His Ala Val Tyr Gly Ile Asn His Pro Leu His Ile Phe Asn Gly Gly			
	50	55	60

114

(A) ORGANISM: *Leishmania major*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

```

GGCACGANAG ATCTTCGTGA AGACGCTGAC CGGCAANACG ATCGCGCTGG AGGTGGAGCC      60
GAGCGACACG ATCGAGAACG TGAAGGCCAA GATCCAGGAC AAGGAGGGCA TCCCGCCGGA      120
CCAGCAGCGC CTGATCTTCG CCGGCAAGCA GCTGGAGGAN GGCCGCACGC TCTCGGACTA      180
CAACATCCAG AAGGAGTCCA CGCTGCACCT GGTGCTGCGC CTGCGCGGCG GCATGCANAT      240
CTTCGTGAAA ACGCTNACCG GCAANACAAT CGCGCTGGAA GTGGAGCCGA ACGACCNATC      300
GAAAACGTGA AGGCCNANAT CCANGACAAG GAAGGCNTCC CGCCGGANCA GCACGCCTGA      360
TCTTCNCCG GCAACCACTT GANGAAGGGC NCACGCTCTC NGACTIONAC ATCCANAAAG      420
GATTCCNCCC TGCACCTTGT TGCTTGCNCC TTGCTCGGGG GGCATGCCNA ATCTTCCTTN      480
AAAACCTCAA CCGGCAANAA CAATCCCCCN CNGAAGTTGG AACCCAACCA NCCCATTENA      540
AAACTTTAAA GGCCNNNATT CCNGAACAAAN GAAGGGCTTC CCCCCGGAC CNNCAANCNC      600
CCTGATTNTT CCCCCGNNN NCANTTTGGA ANGAAGGGCC CCNCCCTCCN CCGAATTNCN      660
ACNTCCCNAA ANGGATTCCC CCCCTNCCCT TGNTTTTTCG GCCNNNNNNC GGCNNCNTNC      720
CNAAATTCCG NCCNAAGGNC CCCANTANAN CNACTTTCCC NTTCCCCCCC NNNNTTTTGC      780
NTAAANTTTT TNCCCCCNNA AANNTCCCN TTNANNTTN AN      822

```

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 146 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Leishmania major*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

```

Gly Thr Ser Pro Cys Leu His Leu Leu Ala Asp Ile Arg Gly Glu Phe
1           5           10           15

```

```

Phe Asn Leu Arg Arg Val Glu Leu Leu Asn Val Ala Gln Gln Ala Gln

```

113

TGCACGGGGT TGCTGTGGCT GCACCTCCTG ACCACTGCCA GCTTTCTTGG CTTGCCTCCC	420
CTCTGCGCCT CCGCTCGTGC CGCTCGTGCC GAATTCGATA TCAAGCTTAT CGATACCGTC	480
NACCTCGAAG GGGGGCCCCG TTACCCATTC GCCCTATANT GAGTCNTATT ACAATTCCTG	540
GCGTCGTTTT ACACGTCGTG ACTGGGAAAA ACCCTGGCGT TCCCCACTTA TCGCCTTGCA	600

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 516 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Leishmania major

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

AGCTGCAGCA GCGCCTAGAC ACCGCCACGC AGCAGCGCGC CGAGCTGGAG GCACGGGTGG	60
CACGGCTGGC CGCGGACCGC GACGAGGCGC GCCAGCAGCT GGCCGCGAAC GCCGAGGAGC	120
TGCAGCAGCG CCTAGACACC GCCACGCAGC AGCGCGCCGA GCTGGAGGCA CGGGTGGCAC	180
GGCTGGCCGC GGACGGCGAC GAGGCCCCGCC AGCAGCTGGC CGCGAACGCC GAGGAGCTGC	240
AGCAGCGCCT AGACACCGCC ACGCAGCAGC GCGCCGAGCT GGAGGCACAG GTGGCACGGC	300
TGGCCGCGAA CGCCGAGGAG CTGCAGCAGC GCCTAGACAC CGCCACGCAG CAGCGCGCCG	360
AGCTGGAGGC ACGGGTGGCA CGGCTGGCCG CGGACCGCGA CGAGGCGCGC CAGCAGCTGG	420
CCGCGAACGC CGAGGAGCTG CAGCAGCGCC TAGACACCGC CACGCAGCAG CGCGCCGAGC	480
TGGARGCACA GGTGGCACGG CTGGCCGCGA AMGCCG	516

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 822 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

NGCACGAGAA GCGCAACTGG CGCATCGCAT CTGTGACTAT CTGCCTGAAC AGGGGCAATN	60
GTTTGTGGT AACAGCCTGG TGGTACGTCT GATTGATNCG CTTNCGCAAN TTCCGGCAGG	120
TTACCCGGTG TACANCAACC GTGGGGCCAN CGGTATCNAC NGGCTGCTTT CGACCGCCGC	180
CGGNGTTCAN CGGGCAANCG GCAAACCGAC GCTGGCGATT GTGGGCGATC TCTCCGCACT	240
TTACGATCTC AACGCNCTGG CGTTATTGCG TCAGGTTTCT GCGCCGCTGG TATTAATTGT	300
GGTGAACAAC AACGGCNGGG CAAAATTTTC TCGCTGTTGC CAACGCCCCC AAAGCNAGCG	360
TGAAGCGTTT CTATCTGATG CCGCAAAACG TCCATTTTGA AACACGCCGC CNCCCATGTT	420
TCGANCTGAA AATATCATCG TCCGCAAAAC TGGCANGAAA CTTNGAAAAC CGCATTTTGC	480
CGACNCCCTG GCNCACGCCC AACCACCCCA CCGGTTGATT GAAAATGGTG GGTTAACGAA	540
NCCNNATGGG TGCCCCAAAN CNCNNCCANC CAAATTTCTG GGCCCAGGTT AAANCCCTTT	600

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 600 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Leishmania major*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

ACGATGACCA TGCCCCGAAG GAGGATGGCC ATGCGCCGAA GAACGATGAC CATGCCCCGA	60
AGGAGGATGG CCATGCGCCG AAGAACGATG ACCATGCCCC GAAGGAGGAT GGCCATGCGC	120
CGAAGAACGA CGGGGATGTG CAGAANAAGA GCGAAGATGG AGACAACGTG GGAGAGGGAG	180
GCAAGGGCAA TGAGGATGGT AACGATGATC AGCCGAAGGA GCACGCTGCC GGCAACTAGT	240
GGGCTGCGTC CGGGCTTGTG TGCGANCCGT GCTCTGCACC CCGCCGCTCG TGCATCCTCG	300
CATGTGGACT GCGTGTGTCT CTCCCGCTTT GTCTCTCTCC CCCACACAGT GGCTGATGCC	360

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TTCAAATTNA TTCCCNACCT NCCNTNNCCA AANNTANCNA ATAATCANNC CCCTNTCANN 540
 ANNTCCCAN C TTACCCTCCN NTNGNNGGGN NNNCCNATTN CCCCAANCCC NCNCTAAATA 600

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 600 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Leishmania major

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

GGCAGGAGCC TCAGTGGAGC TCAATGAAGA TATTGCAGTA TCTTACTCTG GATGGCACTC 60
 AGGTCTCCGG CACGCTGCCG CCCAGTGGA GCGCGATGGC ATCGGTGCGA ATTCTTAACC 120
 TGNAGGGTAC TGAGGTCTCT GGTACGCTGC CGCCTGAGTG GATATCNATG ANCAGGCTGC 180
 AAACTCTGAA TCTGCGGCGC ACGAAANTAT CCGGCACTCT GCCGCCCCGAA TGGANTTCTA 240
 TGAACAGCCT GGAGTACTTT CACCTTTATC TTACTCAGGT CTCCGGCAGC CTGCCGCCCCG 300
 AGTGGAGTGG GATGTCNAAG GCCGCATACT TCTGGCTGGA ATACTGCGAC CTGTCCGGCA 360
 NTCTGCCGCC CNAGTGGTCG TCNATGCCAA AGCTGCGCGG TATCTCACTG ANCGGCAACA 420
 AATTCTTGCG NGTGTNTNCC NGA CTNTGG GATTGAGAAA GGTGGTCCTT GTTGTGGGGC 480
 ATCNAAGGAN CAAACCCCAA NGGGCCNCN AATTGCTTGG GCNTGCTTAA GGANTTGCAC 540
 NAACCAACNC CNCCAAAAAC CCCCCCACC NCNAAANNAC NANCCCCCAC TTAANNCCCN 600

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 600 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Leishmania major

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

GGCACGAGCC CTTGCCTACA TTTGCTCGCC GATATTCGCG GGGAGTTCTT CAATTTGCGT	60
CGCGTAGAAC TGCTCAATGT CGCGCAACAA GCGCAGCTCG TCGTGGCGCA CGAAGGTGAT	120
GGCCAGTCCA GTGCGGCCCA TGC GGCCAGT GCGGCCGATG CGGTGAATGT ACTGCTCAGC	180
CGCGAGCGGC AAATCGTAGC TGAGGACGAG CGAGACGCGC TCCACATCAA TGCCACGCGC	240
CCACAGGTCC GTTGTAATGA NCACGCGGCT GTGTCCATTA CGGAATGCCG CATAATCTCG	300
TCGCGCTCCG CCTGGGGCAT GTCGCCGTGC ATGGCGGACA CAGCGAAATT CTCGCGCGTC	360
ATCTTCTTGG CAAGCTGCTC CACCTTTTTG CGGGTGTTC ANAAAACCAC NGCGTGGGCG	420
ATCGTTAAGC TGTCGTACAA ACTCCATCAA GAAATCGAAT TTGTTTTTCT CTCGTCNAC	480
NGANACAAAN TACTGTTTAA CGCTNTCCAC GGTGATCTCA	520

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 600 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic).

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Leishmania major*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GGCACAAGGT TTTCGGGTGA TCTTCACGCA TGGTGGAGCG CAGATGGGTG AAGTAAATAC	60
GCGGACCGAA CTGCTTGATC ATATCAACCA GATCGTTGTC AGCACGCACG CCGTANGAAC	120
CGGTGCACAT GGTA AAACCG TNTGCCATGC TGTTTACGGT ATCAACCATC CACTGCATAT	180
CTTCAATGGT GGAAACAATG CGCGGCAGGC CGAGGATCCG GCGCGGCTCA TCATNNAGNT	240
NATNAACCAN TCGCAGTCT ANTTCTGCAC TAAACTACAA NTATCGGTNA CATATNATAA	300
GGCCNATTTT CGGTCCAGGA NTATGTNCTN TCAAAATGCC NCGTTANNCA CTCTTAAATG	360
TCTCANGNGN AAANTNGTTC TAAAGGGTGT CCAAAANNTN NTTACCNTTC CCCNCTTACT	420
TCAANANCTC CTCNAATTCC CNGGCCCTTN GACNANNATT TNCTATTAAA ANATANAANN	480

109

Gly Glu Val Cys Pro Ala Asn Trp Lys Lys Gly Ala Pro Thr Met Lys
 180 185 190

Pro Glu Pro Lys Ala Ser Val Glu Gly Tyr Phe Ser Lys Gln
 195 200 205

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 51 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "5' PCR primer (MAPS-1-5'His) to simultaneously amplify MAPS-1 cDNA for both L. major and L. tropica while adding 6 His residues to amino terminal end of encoded protein."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

CAATTACATA TGCATCACCA TCACCATCAC ATGTCCTGCG GTAACGCCAA G

51

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 31 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "3' PCR primer (MAPS-1-3'OR1) to simultaneously amplify MAPS-1 cDNA for both L. major and L. tropica."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

CATGGAATTC TTACTGCTTG CTGAAGTATC C

31

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 520 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Leishmania major

108

AAG CAC GGC GAG GTG TGC CCC GCG AAC TGG AAG AAG GGC GCC CCC ACG 576
 Lys His Gly Glu Val Cys Pro Ala Asn Trp Lys Lys Gly Ala Pro Thr
 175 180 185 190

ATG AAG CCG GAA CCG AAG GCG TCT GTC GAG GGA TAC TTC AGC AAG CAG 624
 Met Lys Pro Glu Pro Lys Ala Ser Val Glu Gly Tyr Phe Ser Lys Gln
 195 200 205

TAAGAATTCC ATG 637

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 206 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Met His His His His His His Met Ser Cys Gly Asn Ala Lys Ile Asn
 1 5 10 15
 Ser Pro Ala Pro Pro Phe Glu Glu Met Ala Leu Met Pro Asn Gly Ser
 20 25 30
 Phe Lys Lys Ile Ser Leu Ser Ala Tyr Lys Gly Lys Trp Val Val Leu
 35 40 45
 Phe Phe Tyr Pro Leu Asp Phe Thr Phe Val Cys Pro Thr Glu Ile Ile
 50 55 60
 Ala Phe Ser Asp Asn Val Ser Arg Phe Asn Glu Leu Asn Cys Glu Val
 65 70 75 80
 Leu Ala Cys Ser Met Asp Ser Glu Tyr Ala His Leu Gln Trp Thr Leu
 85 90 95
 Gln Asp Arg Lys Lys Gly Gly Leu Gly Ala Met Ala Ile Pro Met Leu
 100 105 110
 Ala Asp Lys Thr Lys Ser Ile Ala Arg Ser Tyr Gly Val Leu Glu Glu
 115 120 125
 Ser Gln Gly Val Ala Tyr Arg Gly Leu Phe Ile Ile Asp Pro Arg Gly
 130 135 140
 Met Val Arg Gln Ile Thr Val Asn Asp Met Pro Val Gly Arg Asn Val
 145 150 155 160
 Glu Glu Ala Leu Arg Leu Leu Glu Ala Leu Gln Phe Val Glu Lys His
 165 170 175

107

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Leishmania tropica*

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 7..624

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

TTACAT ATG CAT CAC CAC CAC CAC CAC ATG TCC TGC GGT AAC GCC AAG	48
Met His His His His His His Met Ser Cys Gly Asn Ala Lys	
1 5 10	
ATC AAC TCT CCC GCG CCG CCC TTC GAG GAG ATG GCG CTC ATG CCC AAC	96
Ile Asn Ser Pro Ala Pro Pro Phe Glu Glu Met Ala Leu Met Pro Asn	
15 20 25 30	
GGC AGC TTC AAG AAG ATC AGC CTC TCC GCC TAC AAG GGC AAG TGG GTC	144
Gly Ser Phe Lys Lys Ile Ser Leu Ser Ala Tyr Lys Gly Lys Trp Val	
35 40 45	
GTG CTC TTC TTC TAC CCG CTC GAC TTC ACC TTC GTG TGC CCG ACA GAG	192
Val Leu Phe Phe Tyr Pro Leu Asp Phe Thr Phe Val Cys Pro Thr Glu	
50 55 60	
ATC ATC GCG TTC TCC GAC AAC GTG AGT CGC TTC AAC GAG CTC AAC TGC	240
Ile Ile Ala Phe Ser Asp Asn Val Ser Arg Phe Asn Glu Leu Asn Cys	
65 70 75	
GAG GTC CTC GCG TGC TCG ATG GAC AGC GAG TAC GCG CAC CTG CAG TGG	288
Glu Val Leu Ala Cys Ser Met Asp Ser Glu Tyr Ala His Leu Gln Trp	
80 85 90	
ACG CTG CAG GAC CGC AAG AAG GGC GGC CTC GGG GCC ATG GCG ATC CCA	336
Thr Leu Gln Asp Arg Lys Lys Gly Gly Leu Gly Ala Met Ala Ile Pro	
95 100 105 110	
ATG CTG GCC GAC AAG ACT AAG AGC ATC GCT CGT TCC TAC GGC GTG CTG	384
Met Leu Ala Asp Lys Thr Lys Ser Ile Ala Arg Ser Tyr Gly Val Leu	
115 120 125	
GAG GAG AGC CAG GGC GTG GCC TAC CGC GGT CTC TTC ATC ATC GAC CCC	432
Glu Glu Ser Gln Gly Val Ala Tyr Arg Gly Leu Phe Ile Ile Asp Pro	
130 135 140	
CGT GGC ATG GTG CGT CAG ATC ACC GTC AAC GAC ATG CCG GTG GGC CGC	480
Arg Gly Met Val Arg Gln Ile Thr Val Asn Asp Met Pro Val Gly Arg	
145 150 155	
AAC GTG GAG GAG GCT CTG CGC CTG CTG GAG GCT TTG CAG TTC GTG GAG	528
Asn Val Glu Glu Ala Leu Arg Leu Leu Glu Ala Leu Gln Phe Val Glu	
160 165 170	

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(A) LENGTH: 199 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

```

Met Ser Cys Gly Asn Ala Lys Ile Asn Ser Pro Ala Pro Ser Phe Glu
 1           5           10           15
Glu Val Ala Leu Met Pro Asn Gly Ser Phe Lys Lys Ile Ser Leu Ser
          20           25           30
Ser Tyr Lys Gly Lys Trp Val Val Leu Phe Phe Tyr Pro Leu Asp Phe
          35           40           45
Ser Phe Val Cys Pro Thr Glu Val Ile Ala Phe Ser Asp Ser Val Ser
          50           55           60
Arg Phe Asn Glu Leu Asn Cys Glu Val Leu Ala Cys Ser Ile Asp Ser
          65           70           75           80
Glu Tyr Ala His Leu Gln Trp Thr Leu Gln Asp Arg Lys Lys Gly Gly
          85           90           95
Leu Gly Thr Met Ala Ile Pro Met Leu Ala Asp Lys Thr Lys Ser Ile
          100          105          110
Ala Arg Ser Tyr Gly Val Leu Glu Glu Ser Gln Gly Val Ala Tyr Arg
          115          120          125
Gly Leu Phe Ile Ile Asp Pro His Gly Met Leu Arg Gln Ile Thr Val
          130          135          140
Asn Asp Met Pro Val Gly Arg Ser Val Glu Glu Val Leu Arg Leu Leu
          145          150          155          160
Glu Ala Phe Gln Phe Val Glu Lys His Gly Glu Val Cys Pro Ala Asn
          165          170          175
Trp Lys Lys Gly Ala Pro Thr Met Lys Pro Glu Pro Asn Ala Ser Val
          180          185          190
Glu Gly Tyr Phe Ser Lys Gln
          195

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(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 637 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

105

TTC AAG AAG ATC AGC CTC TCC TCC TAC AAG GGC AAG TGG GTC GTG CTC	149
Phe Lys Lys Ile Ser Leu Ser Ser Tyr Lys Gly Lys Trp Val Val Leu	
30 35 40	
TTC TTC TAC CCG CTC GAC TTT AGC TTC GTG TGC CCG ACA GAG GTC ATC	197
Phe Phe Tyr Pro Leu Asp Phe Ser Phe Val Cys Pro Thr Glu Val Ile	
45 50 55	
GCG TTC TCC GAC AGC GTG AGT CGC TTC AAC GAG CTC AAC TGC GAG GTC	245
Ala Phe Ser Asp Ser Val Ser Arg Phe Asn Glu Leu Asn Cys Glu Val	
60 65 70	
CTC GCG TGC TCG ATA GAC AGC GAG TAC GCG CAC CTG CAG TGG ACG CTG	293
Leu Ala Cys Ser Ile Asp Ser Glu Tyr Ala His Leu Gln Trp Thr Leu	
75 80 85	
CAG GAC CGC AAG AAG GGC GGC CTC GGG ACC ATG GCG ATC CCA ATG CTA	341
Gln Asp Arg Lys Lys Gly Gly Leu Gly Thr Met Ala Ile Pro Met Leu	
90 95 100 105	
GCC GAC AAG ACC AAG AGC ATC GCT CGT TCC TAC GGC GTG CTG GAG GAG	389
Ala Asp Lys Thr Lys Ser Ile Ala Arg Ser Tyr Gly Val Leu Glu Glu	
110 115 120	
AGC CAG GGC GTG GCC TAC CGC GGT CTC TTC ATC ATC GAC CCC CAT GGC	437
Ser Gln Gly Val Ala Tyr Arg Gly Leu Phe Ile Ile Asp Pro His Gly	
125 130 135	
ATG CTG CGT CAG ATC ACC GTC AAT GAC ATG CCG GTG GGC CGC AGC GTG	485
Met Leu Arg Gln Ile Thr Val Asn Asp Met Pro Val Gly Arg Ser Val	
140 145 150	
GAG GAG GTT CTA CGC CTG CTG GAG GCT TTT CAG TTC GTG GAG AAG CAC	533
Glu Glu Val Leu Arg Leu Leu Glu Ala Phe Gln Phe Val Glu Lys His	
155 160 165	
GGC GAG GTG TGC CCC GCG AAC TGG AAG AAG GGC GCC CCC ACG ATG AAG	581
Gly Glu Val Cys Pro Ala Asn Trp Lys Lys Gly Ala Pro Thr Met Lys	
170 175 180 185	
CCG GAA CCG AAT GCG TCT GTC GAG GGA TAC TTC AGC AAG CAG	623
Pro Glu Pro Asn Ala Ser Val Glu Gly Tyr Phe Ser Lys Gln	
190 195	
TAAACCTGTG AGCGTCGCAG GAGTCAGTGT GACCTCACCC GCCTCTGCCA GTGGGTGCGA	683
GAGGGCGTGA GGGATTGTGG GAAGGCTGTT GGATATGATG CAGACAGCGA TGAATGCAAC	743
TCCCACACAC TGGCCCTCCT CAGCCCTCTC CACACAGACA CACGCACGCA TGTG	797

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

104

Arg Leu Ser Asp Ala Cys Ser Val Pro Asn Cys Lys Lys Cys Glu Thr
 180 185 190

Gly Thr Ser Arg Leu Cys Ala Glu Cys Asp Thr Gly Tyr Ser Leu Ser
 195 200 205

Ala Asp Ala Thr Ser Cys Ser Ser Pro Thr Thr Gln Pro Cys Glu Val
 210 215 220

Glu His Cys Asn Thr Cys Val Asn Gly Asp Ser Thr Arg Cys Ala Tyr
 225 230 235 240

Cys Asn Thr Gly Tyr Tyr Val Ser Asp Gly Lys Cys Lys Ala Met Gln
 245 250 255

Gly Cys Tyr Val Ser Asn Cys Ala Gln Cys Met Leu Leu Asp Ser Thr
 260 265 270

Lys Cys Ser Thr Cys Val Lys Gly Tyr Leu Leu Thr Ser Ser Tyr Ser
 275 280 285

Cys Val Ser Gln Lys Val Ile Asn Ser Ala Ala Ala Pro Tyr Ser Leu
 290 295 300

Trp Val Ala Ala Ala Val Leu Leu Thr Ser Phe Ala Met His Leu Ala
 305 310 315 320

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 797 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Leishmania major

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 27..623

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

CTGTACTTTA TTGCCACCAG CCAGCC ATG TCC TGC GGT AAC GCC AAG ATC AAC
 Met Ser Cys Gly Asn Ala Lys Ile Asn
 1 5

53

TCT CCC GCG CCG TCC TTC GAG GAG GTG GCG CTC ATG CCC AAC GGC AGC
 Ser Pro Ala Pro Ser Phe Glu Glu Val Ala Leu Met Pro Asn Gly Ser
 10 15 20 25

101

103

CCACAGTCCC ACAGCGCAGA CACACGTGTT TAAACGGCGC AGGCATCCCT CCCTATCACT 1313
 TCATTTCTCC TAAAGCCACT CACCAAGTCG CACACCGCCC TCCCCCATCG GCCGCCCTTC 1373
 CGGGCGCAGC TGTGCGGAAT GGGTGTGTGC TCGACCTCGT TCCTGGCAGC TCACTCGCAT 1433
 GTGTACAGCC ACTCCAACCA CGAAAGCTCT CTTCTGCGCA CATAAAAAAA AAAAAAAAAA 1493
 AAAAACTCGA GGGGGGGCCC GGTACCCAAA 1523

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 320 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Val Leu Pro Asp Met Thr Cys Ser Leu Thr Gly Leu Gln Cys Thr Asp
 1 5 10 15
 Pro Asn Cys Lys Thr Cys Thr Thr Tyr Gly Gln Cys Thr Asp Cys Asn
 20 25 30
 Asp Gly Tyr Gly Leu Thr Ser Ser Ser Val Cys Val Arg Cys Ser Val
 35 40 45
 Ala Gly Cys Lys Ser Cys Pro Val Asp Ala Asn Val Cys Lys Val Cys
 50 55 60
 Leu Gly Gly Ser Glu Pro Ile Asn Asn Met Cys Pro Cys Thr Asp Pro
 65 70 75 80
 Asn Cys Ala Ser Cys Pro Ser Asp Ala Gly Thr Cys Thr Gln Cys Ala
 85 90 95
 Asn Gly Tyr Gly Leu Val Asp Gly Ala Cys Val Arg Cys Gln Glu Pro
 100 105 110
 Asn Cys Phe Ser Cys Asp Ser Asp Ala Asn Lys Cys Thr Gln Cys Ala
 115 120 125
 Pro Asn Tyr Tyr Leu Thr Pro Leu Leu Thr Cys Ser Pro Val Ala Cys
 130 135 140
 Asn Ile Glu His Cys Met Gln Cys Asp Pro Gln Thr Pro Ser Arg Cys
 145 150 155 160
 Gln Glu Cys Val Ser Pro Tyr Val Val Asp Ser Tyr Asp Gly Leu Cys
 165 170 175

102

Pro Val Ala Cys Asn Ile Glu His Cys Met Gln Cys Asp Pro Gln Thr	
145 150 155	
CCG TCG CGC TGC CAG GAG TGC GTG TCC CCC TAC GTG GTT GAC AGC TAC	529
Pro Ser Arg Cys Gln Glu Cys Val Ser Pro Tyr Val Val Asp Ser Tyr	
160 165 170	
GAC GGC CTC TGC AGG CTC TCC GAT GCC TGC TCC GTG CCC AAC TGC AAG	577
Asp Gly Leu Cys Arg Leu Ser Asp Ala Cys Ser Val Pro Asn Cys Lys	
175 180 185	
AAG TGC GAG ACC GGT ACC TCC AGG CTC TGC GCC GAG TGC GAC ACC GGC	625
Lys Cys Glu Thr Gly Thr Ser Arg Leu Cys Ala Glu Cys Asp Thr Gly	
190 195 200	
TAC AGT CTC TCC GCC GAC GCG ACG AGC TGC AGC AGT CCA ACC ACG CAG	673
Tyr Ser Leu Ser Ala Asp Ala Thr Ser Cys Ser Ser Pro Thr Thr Gln	
205 210 215 220	
CCG TGC GAG GTG GAG CAC TGC AAC ACA TGT GTG AAC GGC GAT AGC ACC	721
Pro Cys Glu Val Glu His Cys Asn Thr Cys Val Asn Gly Asp Ser Thr	
225 230 235	
CGC TGT GCC TAC TGC AAC ACC GGC TAC TAC GTC TCC GAT GGC AAG TGC	769
Arg Cys Ala Tyr Cys Asn Thr Gly Tyr Tyr Val Ser Asp Gly Lys Cys	
240 245 250	
AAG GCC ATG CAG GGC TGC TAC GTG TCG AAC TGC GCG CAG TGC ATG CTG	817
Lys Ala Met Gln Gly Cys Tyr Val Ser Asn Cys Ala Gln Cys Met Leu	
255 260 265	
CTT GAC AGC ACC AAG TGC TCC ACG TGC GTG AAA GGG TAC CTG CTC ACG	865
Leu Asp Ser Thr Lys Cys Ser Thr Cys Val Lys Gly Tyr Leu Leu Thr	
270 275 280	
TCG TCC TAC AGT TGC GTC TCG CAG AAA GTC ATC AAC AGT GCG GCC GCG	913
Ser Ser Tyr Ser Cys Val Ser Gln Lys Val Ile Asn Ser Ala Ala Ala	
285 290 295 300	
CCC TAC TCT CTG TGG GTG GCC GCC GCC GTG CTC CTC ACC TCT TTT GCC	961
Pro Tyr Ser Leu Trp Val Ala Ala Ala Val Leu Leu Thr Ser Phe Ala	
305 310 315	
ATG CAC CTA GCA TAGTGCGCAG CGGCATGCGA ACAACCCAC TCTCATTCTC	1013
Met His Leu Ala	
320	
CAACATGTGC ATACACACAC ACACAGACAG CGGGGCAGCA CCCCTCCCC ACACACACAC	1073
ACGCACTTCC CCCTTGTCTT GTTCTTCTTT CCTCGTTTCG CTTTCTTTCT CTCGTGCGCT	1133
GGCGCCGGCC TCCTGCACGT CGCTCCCCCTC CCCCTAACCT CTATTCTCTC TCTCTCTCTC	1193
TCTCGCCGGC ATCATTGCTT CTTACCCCTTT TCTGATCCTT GCTCGCGTGG GCGGACACTG	1253

101

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1523 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Leishmania major*

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 14..973

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GAATTCGGCA CGA GTG CTG CCC GAC ATG ACA TGC TCG CTG ACC GGA CTT	49
Val Leu Pro Asp Met Thr Cys Ser Leu Thr Gly Leu	
1 5 10	
CAG TGC ACA GAC CCG AAC TGC AAG ACC TGC ACA ACT TAC GGT CAG TGC	97
Gln Cys Thr Asp Pro Asn Cys Lys Thr Cys Thr Thr Tyr Gly Gln Cys	
15 20 25	
ACA GAC TGC AAC GAC GGC TAC GGT CTC ACC TCC TCC AGC GTT TGC GTG	145
Thr Asp Cys Asn Asp Gly Tyr Gly Leu Thr Ser Ser Ser Val Cys Val	
30 35 40	
CGC TGC AGT GTA GCG GGC TGC AAG AGC TGC CCC GTC GAC GCT AAC GTC	193
Arg Cys Ser Val Ala Gly Cys Lys Ser Cys Pro Val Asp Ala Asn Val	
45 50 55 60	
TGC AAA GTG TGT CTC GGC GGC AGC GAG CCG ATC AAC AAT ATG TGC CCC	241
Cys Lys Val Cys Leu Gly Gly Ser Glu Pro Ile Asn Asn Met Cys Pro	
65 70 75	
TGC ACC GAC CCC AAC TGC GCC AGC TGC CCC AGC GAC GCT GGC ACG TGC	289
Cys Thr Asp Pro Asn Cys Ala Ser Cys Pro Ser Asp Ala Gly Thr Cys	
80 85 90	
ACT CAG TGC GCG AAC GGC TAC GGT CTC GTG GAC GGC GCC TGT GTG AGA	337
Thr Gln Cys Ala Asn Gly Tyr Gly Leu Val Asp Gly Ala Cys Val Arg	
95 100 105	
TGC CAG GAG CCC AAC TGC TTC AGC TGC GAC AGC GAC GCG AAT AAG TGC	385
Cys Gln Glu Pro Asn Cys Phe Ser Cys Asp Ser Asp Ala Asn Lys Cys	
110 115 120	
ACA CAA TGT GCG CCG AAC TAC TAC CTC ACC CCG CTC TTG ACC TGC TCC	433
Thr Gln Cys Ala Pro Asn Tyr Tyr Leu Thr Pro Leu Leu Thr Cys Ser	
125 130 135 140	
CCG GTG GCC TGC AAC ATC GAG CAC TGC ATG CAG TGC GAC CCA CAG ACG	481

100

TGTGTAGCCA TGGCTTCAGG AGAGAAAACA AAATACAAGG AAAGGCAATA TGTAACATATG 783
 GGGTTCCCTT TTTTACTATG CAAAGTTTTT ATAACCTCTG ATCGGCAAAA ACAACAACAA 843
 CCGCCATACA CCAAGAGCAA ATGCTTTCTT CTGCGGACTG TGCTTCTGTT TTTTTTTATG 903
 AAGGAGTGAC TCGCGCGATG AAAAGTGTGT GCGTGGGAGA TGTATTTCTT TTTTTTGTTT 963
 ATAGTGGCGA CAGCTCACTG TTGACGATGA CAAAAAAAAA AAAAAAAAAA CTCGAG 1019

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 151 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Met Ser Ser Glu Arg Thr Phe Ile Ala Val Lys Pro Asp Gly Val Gln
 1 5 10 15
 Arg Gly Leu Val Gly Glu Ile Ile Ala Arg Phe Glu Arg Lys Gly Tyr
 20 25 30
 Lys Leu Val Ala Leu Lys Ile Leu Gln Pro Thr Thr Glu Gln Ala Gln
 35 40 45
 Gly His Tyr Lys Asp Leu Cys Ser Lys Pro Phe Phe Pro Ala Leu Val
 50 55 60
 Lys Tyr Phe Ser Ser Gly Pro Ile Val Cys Met Val Trp Glu Gly Lys
 65 70 75 80
 Asn Val Val Lys Ser Gly Arg Val Leu Leu Gly Ala Thr Asn Pro Ala
 85 90 95
 Asp Ser Gln Pro Gly Thr Ile Arg Gly Asp Phe Ala Val Asp Val Gly
 100 105 110
 Arg Asn Val Cys His Gly Ser Asp Ser Val Glu Ser Ala Glu Arg Glu
 115 120 125
 Ile Ala Phe Trp Phe Lys Ala Asp Glu Ile Ala Ser Trp Thr Ser His
 130 135 140
 Ser Val Ser Gln Ile Tyr Glu
 145 150

(2) INFORMATION FOR SEQ ID NO:21:

99

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 71..523

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GAATTCGGCA CGAGGTTTCT GTACTTTATT GCTTCAGCC TTTATTCACT CTTGATTTC	60
CTCTAACACC ATG TCC TCC GAG CGC ACC TTT ATT GCC GTC AAG CCG GAC	109
Met Ser Ser Glu Arg Thr Phe Ile Ala Val Lys Pro Asp	
1 5 10	
GGC GTG CAG CGC GGC CTC GTT GGC GAG ATC ATC GCC CGC TTC GAG CGC	157
Gly Val Gln Arg Gly Leu Val Gly Glu Ile Ile Ala Arg Phe Glu Arg	
15 20 25	
AAG GGC TAC AAG CTC GTC GCC TTG AAG ATA CTG CAG CCG ACG ACG GAG	205
Lys Gly Tyr Lys Leu Val Ala Leu Lys Ile Leu Gln Pro Thr Thr Glu	
30 35 40 45	
CAG GCC CAG GGT CAC TAT AAG GAC CTT TGC TCC AAG CCG TTT TTC CCG	253
Gln Ala Gln Gly His Tyr Lys Asp Leu Cys Ser Lys Pro Phe Phe Pro	
50 55 60	
GCC CTT GTG AAG TAC TTC TCC TCT GGC CCG ATC GTG TGT ATG GTG TGG	301
Ala Leu Val Lys Tyr Phe Ser Ser Gly Pro Ile Val Cys Met Val Trp	
65 70 75	
GAG GGT AAG AAC GTG GTG AAG AGC GGC CGC GTG CTG CTC GGC GCG ACG	349
Glu Gly Lys Asn Val Val Lys Ser Gly Arg Val Leu Leu Gly Ala Thr	
80 85 90	
AAC CCG GCC GAC TCA CAG CCC GGC ACG ATC CGT GGC GAC TTT GCC GTG	397
Asn Pro Ala Asp Ser Gln Pro Gly Thr Ile Arg Gly Asp Phe Ala Val	
95 100 105	
GAT GTG GGC CGC AAC GTG TGC CAC GGG TCC GAC TCT GTG GAG AGC GCG	445
Asp Val Gly Arg Asn Val Cys His Gly Ser Asp Ser Val Glu Ser Ala	
110 115 120 125	
GAG CGC GAG ATC GCC TTT TGG TTC AAG GCG GAT GAG ATC GCG AGC TGG	493
Glu Arg Glu Ile Ala Phe Trp Phe Lys Ala Asp Glu Ile Ala Ser Trp	
130 135 140	
ACG TCG CAC TCC GTG TCC CAG ATC TAT GAG TAACGGTGAT TCGGACACG	543
Thr Ser His Ser Val Ser Gln Ile Tyr Glu	
145 150	
CTTTGAGGAC GTAGCTGTAC CCCCATGAA TTCTTCTCTG AAAACCACAT CATAAGCCTC	603
TTAAGAGGTT ATTTTCTTG ATCGATGCCC GGTGGTGACC AGCACCATTC CTTTATCGGA	663
TTCACTCACA CTCCTAGCGA ATCATGTAGT GCGGTGAGAG TGGGCTCTGG AGGAGACTGT	723

98

Lys His Gly Leu Glu Val Ile Tyr Met Ile Glu Pro Ile Asp Glu Tyr
 515 520 525
 Cys Val Gln Gln Leu Lys Glu Phe Glu Gly Lys Thr Leu Val Ser Val
 530 535 540
 Thr Lys Glu Gly Leu Glu Leu Pro Glu Asp Glu Glu Glu Lys Lys Lys
 545 550 555 560
 Gln Glu Glu Lys Lys Thr Lys Phe Glu Asn Leu Cys Lys Ile Met Lys
 565 570 575
 Asp Ile Leu Glu Lys Lys Val Glu Lys Val Val Val Ser Asn Arg Leu
 580 585 590
 Val Thr Ser Pro Cys Cys Leu Val Thr Ser Thr Tyr Gly Trp Thr Ala
 595 600 605
 Asn Met Glu Arg Ile Met Lys Ala Gln Ala Leu Arg Asp Asn Ser Thr
 610 615 620
 Met Gly Tyr Met Ala Ala Lys Lys His Leu Glu Ile Asn Pro Asp His
 625 630 635 640
 Ser Ile Ile Glu Thr Leu Arg Gln Lys Ala Glu Ala Asp Lys Asn Asp
 645 650 655
 Lys Ser Val Lys Asp Leu Val Ile Leu Leu Tyr Glu Thr Ala Leu Leu
 660 665 670
 Ser Ser Gly Phe Ser Leu Glu Asp Pro Gln Thr His Ala Asn Arg Ile
 675 680 685
 Tyr Arg Met Ile Lys Leu Gly Leu Gly Ile Asp Glu Asp Asp Pro Thr
 690 695 700
 Ala Asp Asp Thr Ser Ala Ala Val Thr Glu Glu Met Pro Pro Leu Glu
 705 710 715 720
 Gly Asp Asp Asp Thr Ser Arg Met Glu Glu Val Asp
 725 730

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1019 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Leishmania major*

97

Glu Arg Asp Lys Glu Val Ser Asp Asp Glu Ala Glu Glu Lys Glu Asp
225 230 235 240

Lys Glu Glu Glu Lys Glu Lys Glu Glu Lys Glu Ser Glu Asp Lys Pro
245 250 255

Glu Ile Glu Asp Val Gly Ser Asp Glu Glu Asp Glu Lys Lys Asp Gly
260 265 270

Asp Lys Lys Lys Lys Lys Lys Ile Lys Glu Lys Tyr Ile Asp Lys Glu
275 280 285

Glu Leu Asn Lys Thr Lys Pro Ile Trp Thr Arg Asn Pro Asp Asp Ile
290 295 300

Thr Asn Glu Glu Tyr Gly Glu Phe Tyr Lys Ser Leu Thr Asn Asp Trp
305 310 315 320

Glu Asp His Leu Ala Val Lys His Phe Ser Val Glu Gly Gln Leu Glu
325 330 335

Phe Arg Ala Leu Leu Phe Val Pro Arg Arg Ala Pro Phe Asp Leu Phe
340 345 350

Glu Asn Arg Lys Lys Lys Asn Asn Ile Lys Leu Tyr Val Arg Arg Val
355 360 365

Phe Ile Met Asp Asn Cys Glu Glu Leu Ile Pro Glu Tyr Leu Asn Phe
370 375 380

Ile Arg Gly Val Val Asp Ser Glu Asp Leu Pro Leu Asn Ile Ser Arg
385 390 395 400

Glu Met Leu Gln Gln Ser Lys Ile Leu Lys Val Ile Arg Lys Asn Leu
405 410 415

Val Lys Lys Cys Leu Glu Leu Phe Thr Glu Leu Ala Glu Asp Lys Glu
420 425 430

Asn Tyr Lys Lys Phe Tyr Glu Gln Phe Ser Lys Asn Ile Lys Leu Gly
435 440 445

Ile His Glu Asp Ser Gln Asn Arg Lys Lys Leu Ser Glu Leu Leu Arg
450 455 460

Tyr Tyr Thr Ser Ala Ser Gly Asp Glu Met Val Ser Leu Lys Asp Tyr
465 470 475 480

Cys Thr Arg Met Lys Glu Asn Gln Lys His Ile Tyr Tyr Ile Thr Gly
485 490 495

Glu Thr Lys Asp Gln Val Ala Asn Ser Ala Phe Val Glu Arg Leu Arg
500 505 510

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 732 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

```

Met Pro Glu Glu Thr Gln Thr Gln Asp Gln Pro Met Glu Glu Glu Glu
1           5           10           15
Val Glu Thr Phe Ala Phe Gln Ala Glu Ile Ala Gln Leu Met Ser Leu
20           25           30
Ile Ile Asn Thr Phe Tyr Ser Asn Lys Glu Ile Phe Leu Arg Glu Leu
35           40           45
Ile Ser Asn Ser Ser Asp Ala Leu Asp Lys Ile Arg Tyr Glu Ser Leu
50           55           60
Thr Asp Pro Ser Lys Leu Asp Ser Gly Lys Glu Leu His Ile Asn Leu
65           70           75           80
Ile Pro Asn Lys Gln Asp Arg Ala Leu Thr Ile Val Asp Thr Gly Ile
85           90           95
Gly Met Thr Lys Ala Asp Leu Ile Asn Asn Leu Gly Thr Ile Ala Lys
100          105          110
Ser Gly Thr Lys Ala Phe Met Glu Ala Leu Gln Ala Gly Ala Asp Ile
115          120          125
Ser Met Ile Gly Gln Phe Gly Val Gly Phe Tyr Ser Ala Tyr Leu Val
130          135          140
Ala Glu Lys Val Thr Val Ile Thr Lys His Asn Asp Asp Glu Gln Tyr
145          150          155          160
Ala Trp Glu Ser Ser Ala Gly Gly Ser Phe Thr Val Arg Thr Asp Thr
165          170          175
Gly Glu Pro Met Gly Arg Gly Thr Lys Val Ile Leu His Leu Lys Glu
180          185          190
Asp Gln Thr Glu Tyr Leu Glu Glu Arg Arg Ile Lys Glu Ile Val Lys
195          200          205
Lys His Ser Gln Phe Ile Gly Tyr Pro Ile Thr Leu Phe Val Glu Lys
210          215          220

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95

Gly	Lys	Asn	Val	Lys	Leu	Gly	Ile	His	Glu	Asp	Ser	Ala	Asn	Arg	Lys	420	425	430
Lys	Leu	Met	Glu	Leu	Leu	Arg	Phe	His	Ser	Ser	Glu	Ser	Gly	Glu	Asp	435	440	445
Met	Thr	Thr	Leu	Lys	Asp	Tyr	Val	Thr	Arg	Met	Lys	Glu	Gly	Gln	Lys	450	455	460
Cys	Ile	Tyr	Tyr	Val	Thr	Gly	Asp	Ser	Lys	Lys	Lys	Leu	Glu	Thr	Ser	465	470	475
Pro	Phe	Ile	Glu	Gln	Ala	Arg	Arg	Arg	Gly	Phe	Glu	Val	Leu	Phe	Met	485	490	495
Thr	Glu	Pro	Ile	Asp	Glu	Tyr	Val	Met	Gln	Gln	Val	Lys	Asp	Phe	Glu	500	505	510
Asp	Lys	Lys	Phe	Ala	Cys	Leu	Thr	Lys	Glu	Gly	Val	His	Phe	Glu	Glu	515	520	525
Thr	Glu	Glu	Glu	Lys	Lys	Gln	Arg	Glu	Glu	Glu	Lys	Thr	Ala	Tyr	Glu	530	535	540
Arg	Leu	Cys	Lys	Ala	Met	Lys	Asp	Val	Leu	Gly	Asp	Lys	Val	Glu	Lys	545	550	555
Val	Val	Val	Ser	Glu	Arg	Leu	Ala	Thr	Ser	Pro	Cys	Ile	Leu	Val	Thr	565	570	575
Ser	Glu	Phe	Gly	Trp	Ser	Ala	His	Met	Glu	Gln	Ile	Met	Arg	Asn	Gln	580	585	590
Ala	Leu	Arg	Asp	Ser	Ser	Met	Ser	Ala	Tyr	Met	Met	Ser	Lys	Lys	Thr	595	600	605
Met	Glu	Ile	Asn	Pro	Ala	His	Pro	Ile	Val	Lys	Glu	Leu	Lys	Arg	Arg	610	615	620
Val	Glu	Ala	Asp	Glu	Asn	Asp	Lys	Ala	Val	Lys	Asp	Leu	Val	Tyr	Leu	625	630	635
Leu	Phe	Asp	Thr	Ala	Leu	Leu	Thr	Ser	Gly	Phe	Thr	Leu	Asp	Asp	Pro	645	650	655
Thr	Ser	Tyr	Ala	Glu	Arg	Ile	His	Arg	Met	Ile	Lys	Leu	Gly	Leu	Ser	660	665	670
Leu	Asp	Asp	Glu	Asp	Asn	Gly	Asn	Glu	Glu	Ala	Glu	Pro	Ala	Ala	Ala	675	680	685
Val	Pro	Ala	Glu	Pro	Val	Ala	Gly	Thr	Ser	Ser	Met	Glu	Gln	Val	Asp	690	695	700

Val Ala Asp Arg Val Thr Val Val Ser Lys Asn Asn Asp Asp Glu Ala
 130 135 140
 Tyr Thr Trp Glu Ser Ser Ala Gly Gly Thr Phe Thr Val Thr Pro Thr
 145 150 155 160
 Pro Asp Cys Asp Leu Lys Arg Gly Thr Arg Ile Val Leu His Leu Lys
 165 170 175
 Glu Asp Gln Gln Glu Tyr Leu Glu Glu Arg Arg Leu Lys Asp Leu Ile
 180 185 190
 Lys Lys His Ser Glu Phe Ile Gly Tyr Asp Ile Glu Leu Met Val Glu
 195 200 205
 Lys Ala Thr Glu Lys Glu Val Thr Asp Glu Asp Glu Asp Glu Ala Ala
 210 215 220
 Ala Thr Lys Asn Glu Glu Gly Glu Glu Pro Lys Val Glu Glu Val Lys
 225 230 235 240
 Asp Asp Ala Glu Glu Gly Glu Lys Lys Lys Lys Thr Lys Lys Val Lys
 245 250 255
 Glu Val Thr Gln Glu Phe Val Val Gln Asn Lys His Lys Pro Leu Trp
 260 265 270
 Thr Arg Asp Pro Lys Asp Val Thr Lys Glu Glu Tyr Ala Ala Phe Tyr
 275 280 285
 Lys Ala Ile Ser Asn Asp Trp Glu Glu Pro Leu Ser Thr Lys His Phe
 290 295 300
 Ser Val Glu Gly Gln Leu Glu Phe Arg Ala Ile Leu Phe Val Pro Lys
 305 310 315 320
 Arg Ala Pro Phe Asp Met Phe Glu Pro Ser Lys Lys Arg Asn Asn Ile
 325 330 335
 Lys Leu Tyr Val Arg Arg Val Phe Ile Met Asp Asn Cys Glu Asp Leu
 340 345 350
 Cys Pro Glu Trp Leu Ala Phe Val Arg Gly Val Val Asp Ser Glu Asp
 355 360 365
 Leu Pro Leu Asn Ile Ser Arg Glu Asn Leu Gln Gln Asn Lys Ile Leu
 370 375 380
 Lys Val Ile Arg Lys Asn Ile Val Lys Lys Ala Leu Glu Leu Phe Glu
 385 390 395 400
 Glu Ile Ala Glu Asn Lys Glu Asp Tyr Lys Lys Phe Tyr Glu Gln Phe
 405 410 415

93

Asn Pro Lys His Pro Ile Ile Lys Glu Leu Arg Arg Arg Val Glu Ala
 610 615 620
 Asp Glu Asn Asp Lys Ala Val Lys Asp Leu Val Phe Leu Leu Phe Asp
 625 630 635 640
 Thr Ser Leu Leu Thr Ser Gly Phe Gln Leu Glu Asp Pro Thr Tyr Ala
 645 650 655
 Glu Arg Ile Asn Arg Met Ile Lys Leu Gly Leu Ser Leu Asp Glu Glu
 660 665 670
 Glu Glu Glu Glu Ala Val Glu Ala Ala Val Ala Glu Thr Ala Pro Ala
 675 680 685
 Glu Val Thr Ala Gly Thr Ser Ser Met Glu Leu Val Asp
 690 695 700

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 704 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Met Thr Glu Thr Phe Ala Phe Gln Ala Glu Ile Asn Gln Leu Met Ser
 1 5 10 15
 Leu Ile Ile Asn Thr Phe Tyr Ser Asn Lys Glu Ile Phe Leu Arg Glu
 20 25 30
 Leu Ile Ser Asn Ala Ser Asp Ala Cys Asp Lys Ile Arg Tyr Gln Ser
 35 40 45
 Leu Thr Asn Gln Ala Val Leu Gly Asp Glu Ser His Leu Arg Ile Arg
 50 55 60
 Val Val Pro Asp Lys Ala Asn Lys Thr Leu Thr Val Glu Asp Thr Gly
 65 70 75 80
 Ile Gly Met Thr Lys Ala Glu Leu Val Asn Asn Leu Gly Thr Ile Ala
 85 90 95
 Arg Ser Gly Thr Lys Ala Phe Met Glu Ala Leu Glu Ala Gly Gly Asp
 100 105 110
 Met Ser Met Ile Gly Gln Phe Gly Val Gly Phe Tyr Ser Ala Tyr Leu
 115 120 125

Phe Asp Met Leu Glu Pro Asn Lys Lys Arg Asn Asn Ile Lys Leu Tyr
 325 330 335
 Val Arg Arg Val Phe Ile Met Asp Asn Cys Glu Asp Leu Cys Pro Asp
 340 345 350
 Trp Leu Gly Phe Val Lys Gly Val Val Asp Ser Glu Asp Leu Pro Leu
 355 360 365
 Asn Ile Ser Arg Glu Asn Leu Gln Gln Asn Lys Ile Leu Lys Val Ile
 370 375 380
 Arg Lys Asn Ile Val Lys Lys Cys Leu Glu Met Phe Glu Glu Val Ala
 385 390 395 400
 Glu Asn Lys Glu Asp Tyr Lys Gln Phe Tyr Glu Gln Phe Gly Lys Asn
 405 410 415
 Ile Lys Leu Gly Ile His Glu Asp Thr Ala Asn Arg Lys Lys Leu Met
 420 425 430
 Glu Leu Leu Arg Phe Tyr Ser Thr Glu Ser Gly Glu Val Met Thr Thr
 435 440 445
 Leu Lys Asp Tyr Val Thr Arg Met Lys Ala Glu Gln Asn Ser Ile Tyr
 450 455 460
 Tyr Ile Thr Gly Asp Ser Lys Lys Lys Leu Glu Ser Ser Pro Phe Ile
 465 470 475 480
 Glu Gln Ala Lys Arg Arg Gly Phe Glu Val Leu Phe Met Thr Glu Pro
 485 490 495
 Tyr Asp Glu Tyr Val Met Gln Gln Val Lys Asp Phe Glu Asp Lys Lys
 500 505 510
 Phe Ala Cys Leu Thr Lys Glu Gly Val His Phe Glu Glu Ser Glu Glu
 515 520 525
 Glu Lys Lys Gln Arg Glu Glu Glu Lys Ala Thr Cys Glu Lys Leu Cys
 530 535 540
 Lys Thr Met Lys Glu Val Leu Gly Asp Lys Val Glu Lys Val Thr Val
 545 550 555 560
 Ser Glu Arg Leu Ser Thr Ser Pro Cys Ile Leu Val Thr Ser Glu Phe
 565 570 575
 Gly Trp Ser Ala His Met Glu Gln Met Met Arg Asn Gln Ala Leu Arg
 580 585 590
 Asp Ser Ser Met Ala Gln Tyr Met Met Ser Lys Lys Thr Met Glu Leu
 595 600 605

91

20	25	30
Val Ile Ser Asn Ala Ser Asp Ala Cys Asp Lys Ile Arg Tyr Gln Ser		
35	40	45
Leu Thr Asp Pro Ala Val Leu Gly Asp Ala Thr Arg Leu Cys Val Arg		
50	55	60
Val Val Pro Asp Lys Glu Asn Lys Thr Leu Thr Val Glu Asp Asn Gly		
65	70	75
Ile Gly Met Thr Lys Ala Asp Leu Val Asn Asn Leu Gly Thr Ile Ala		
85	90	95
Arg Ser Gly Thr Lys Ala Phe Met Glu Ala Leu Glu Ala Gly Ala Asp		
100	105	110
Met Ser Met Ile Gly Gln Phe Gly Val Gly Phe Tyr Ser Ala Tyr Leu		
115	120	125
Val Ala Asp Arg Val Thr Val Thr Ser Lys Asn Asn Ser Asp Glu Val		
130	135	140
Tyr Val Trp Glu Ser Ser Ala Gly Gly Thr Phe Thr Ile Thr Ser Ala		
145	150	155
Pro Glu Ser Asp Met Lys Leu Pro Ala Arg Ile Thr Leu His Leu Lys		
165	170	175
Glu Asp Gln Leu Glu Tyr Leu Glu Ala Arg Arg Leu Lys Glu Leu Ile		
180	185	190
Lys Lys His Ser Glu Phe Ile Gly Tyr Asp Ile Glu Leu Met Val Glu		
195	200	205
Lys Thr Thr Glu Lys Glu Val Thr Asp Glu Asp Glu Glu Glu Ala Lys		
210	215	220
Lys Ala Asp Glu Asp Gly Glu Glu Pro Lys Val Glu Glu Val Thr Glu		
225	230	235
Gly Glu Glu Asp Lys Lys Lys Lys Thr Lys Lys Val Lys Glu Val Thr		
245	250	255
Lys Glu Tyr Glu Val Gln Asn Lys His Lys Pro Leu Trp Thr Arg Asp		
260	265	270
Pro Lys Asp Val Thr Lys Glu Glu Tyr Ala Ala Phe Tyr Lys Ala Ile		
275	280	285
Ser Asn Asp Trp Glu Asp Pro Pro Ala Thr Lys His Phe Ser Val Glu		
290	295	300
Gly Gln Leu Glu Phe Arg Ala Ile Met Phe Val Pro Lys Arg Ala Pro		
305	310	315
		320

90

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Lys Val Phe Asp Glu
 1 5

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GGATCCATGG TCAAGTCCCA CTACATCTGC

30

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 33 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GAATTCAGAC CGGATAGAAA TAAGCCAATG AAA

33

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 701 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Met Thr Glu Thr Phe Ala Phe Gln Ala Glu Ile Asn Gln Leu Met Ser
 1 5 10 15

Leu Ile Ile Asn Thr Phe Tyr Ser Asn Lys Glu Ile Phe Leu Arg Asp

89

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 6
- (D) OTHER INFORMATION: /note= "Where Xaa is either a Leu or Lys Residue"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Xaa Gln Xaa Pro Gln Xaa Val Phe Asp Glu Xaa Xaa
1 5 10

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: -
- (B) LOCATION: 11
- (D) OTHER INFORMATION: /note= "Where n is inosine"

(ix) FEATURE:

- (A) NAME/KEY: -
- (B) LOCATION: 17
- (D) OTHER INFORMATION: /note= "Where n is inosine"

(ix) FEATURE:

- (A) NAME/KEY: -
- (B) LOCATION: 20
- (D) OTHER INFORMATION: /note= "Where n is inosine"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GGAATCCCC NCAGCTNGTN TTCGAC

26

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

88

	165		170		175
Leu Asp Glu Ala Asp Glu Met Leu Ser Gln Gly Phe Ala Asp Gln Ile	180		185		190
Tyr Glu Ile Phe Arg Phe Leu Pro Lys Asp Ile Gln Val Ala Leu Phe	195		200		205
Ser Ala Thr Met Pro Glu Glu Val Leu Glu Leu Thr Lys Lys Phe Met	210		215		220
Arg Asp Pro Val Arg Ile Leu Val Lys Arg Glu Ser Leu Thr Leu Glu	225		230		235 240
Gly Ile Lys Gln Phe Phe Ile Ala Val Glu Glu Glu His Lys Leu Asp		245		250	255
Thr Leu Met Asp Leu Tyr Glu Thr Val Ser Ile Ala Gln Ser Val Ile	260		265		270
Phe Ala Asn Thr Arg Arg Lys Val Asp Trp Ile Ala Glu Lys Leu Asn	275		280		285
Gln Ser Asn His Thr Val Ser Ser Met His Ala Glu Met Pro Lys Ser	290		295		300
Asp Arg Glu Arg Val Met Asn Thr Phe Arg Ser Gly Ser Ser Arg Val	305		310		315 320
Leu Val Thr Thr Asp Leu Val Ala Arg Gly Ile Asp Val His His Val		325		330	335
Asn Ile Val Ile Asn Phe Asp Leu Pro Thr Asn Lys Glu Asn Tyr Leu	340		345		350
His Arg Ile Gly Arg Gly Gly Arg Tyr Gly Val Lys Gly Val Ala Ile	355		360		365
Asn Phe Val Thr Glu Lys Asp Val Glu Leu Leu His Glu Ile Glu Gly	370		375		380
His Tyr His Thr Gln Ile Asp Glu Leu Pro Val Asp Phe Ala Ala Tyr	385		390		395 400
Leu Gly Glu					

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

AGAGAACGCA CATCGTAACA CAGCCACGCG AACGATAGTA AGGGCGTGCG GCGGCGTTCC 1426
 CCTCCTCCTG CCAGCGGCCC CCCTCCGAG CGCTTCTCTT TTGAGAGGGG GGCAGGGGGA 1486
 GCGCTGCGC CTGGCTGGAT GTGTGCTTGA GCTTGCATTC CGTCAAGCAA GTGCTTTGTT 1546
 TTAATTATGC GCGCCGTTTT GTTGCTCGTC CCTTTCGTTG GTGTTTTTTC GGCCGAAACG 1606
 GCGTTTAAAG CA 1618

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 403 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Ser Gln Gln Asp Arg Val Ala Pro Gln Asp Gln Asp Ser Phe Leu
 1 5 10 15
 Asp Asp Gln Pro Gly Val Arg Pro Ile Pro Ser Phe Asp Asp Met Pro
 20 25 30
 Leu His Gln Asn Leu Leu Arg Gly Ile Tyr Ser Tyr Gly Phe Glu Lys
 35 40 45
 Pro Ser Ser Ile Gln Gln Arg Ala Ile Ala Pro Phe Thr Arg Gly Gly
 50 55 60
 Asp Ile Ile Ala Gln Ala Gln Ser Gly Thr Gly Lys Thr Gly Ala Phe
 65 70 75 80
 Ser Ile Gly Leu Leu Gln Arg Leu Asp Phe Arg His Asn Leu Ile Gln
 85 90 95
 Gly Leu Val Leu Ser Pro Thr Arg Glu Leu Ala Leu Gln Thr Ala Glu
 100 105 110
 Val Ile Ser Arg Ile Gly Glu Phe Leu Ser Asn Ser Ala Lys Phe Cys
 115 120 125
 Glu Thr Phe Val Gly Gly Thr Arg Val Gln Asp Asp Leu Arg Lys Leu
 130 135 140
 Gln Ala Gly Val Val Val Ala Val Gly Thr Pro Gly Arg Val Ser Asp
 145 150 155 160
 Val Ile Lys Arg Gly Ala Leu Arg Thr Glu Ser Leu Arg Val Leu Val

86

GAG ATC TTC CGC TTC CTG CCG AAG GAC ATC CAG GTC GCG CTC TTC TCC Glu Ile Phe Arg Phe Leu Pro Lys Asp Ile Gln Val Ala Leu Phe Ser 195 200 205	741
GCC ACG ATG CCG GAG GAG GTG CTG GAG CTG ACA AAG AAG TTC ATG CGC Ala Thr Met Pro Glu Glu Val Leu Glu Leu Thr Lys Lys Phe Met Arg 210 215 220 225	789
GAC CCC GTA CGC ATT CTC GTG AAG CGC GAG AGC CTG ACG CTG GAG GGC Asp Pro Val Arg Ile Leu Val Lys Arg Glu Ser Leu Thr Leu Glu Gly 230 235 240	837
ATC AAG CAG TTC TTC ATC GCC GTC GAG GAG GAG CAC AAG CTG GAC ACG Ile Lys Gln Phe Phe Ile Ala Val Glu Glu Glu His Lys Leu Asp Thr 245 250 255	885
CTG ATG GAC CTG TAC GAG ACC GTG TCC ATC GCG CAG TCC GTC ATC TTC Leu Met Asp Leu Tyr Glu Thr Val Ser Ile Ala Gln Ser Val Ile Phe 260 265 270	933
GCC AAC ACC CGC CGC AAG GTG GAC TGG ATC GCC GAG AAG CTG AAT CAG Ala Asn Thr Arg Arg Lys Val Asp Trp Ile Ala Glu Lys Leu Asn Gln 275 280 285	981
AGC AAC CAC ACC GTC AGC AGC ATG CAC GCC GAG ATG CCC AAG AGC GAC Ser Asn His Thr Val Ser Ser Met His Ala Glu Met Pro Lys Ser Asp 290 295 300 305	1029
CGC GAG CGC GTC ATG AAC ACC TTC CGC AGC GGC AGC TCC CGC GTG CTC Arg Glu Arg Val Met Asn Thr Phe Arg Ser Gly Ser Ser Arg Val Leu 310 315 320	1077
GTA ACG ACC GAC CTC GTG GCC CGC GGC ATC GAC GTG CAC CAC GTG AAC Val Thr Thr Asp Leu Val Ala Arg Gly Ile Asp Val His His Val Asn 325 330 335	1125
ATC GTC ATC AAC TTC GAC CTG CCG ACG AAC AAG GAG AAC TAC CTG CAC Ile Val Ile Asn Phe Asp Leu Pro Thr Asn Lys Glu Asn Tyr Leu His 340 345 350	1173
CGC ATT GGC CGC GGC GGC CGC TAC GGC GTA AAG GGT GTT GCC ATC AAC Arg Ile Gly Arg Gly Gly Arg Tyr Gly Val Lys Gly Val Ala Ile Asn 355 360 365	1221
TTC GTG ACG GAG AAA GAC GTG GAG CTG CTG CAC GAG ATC GAG GGG CAC Phe Val Thr Glu Lys Asp Val Glu Leu Leu His Glu Ile Glu Gly His 370 375 380 385	1269
TAC CAC ACG CAG ATC GAT GAG CTC CCG GTG GAC TTT GCC GCC TAC CTC Tyr His Thr Gln Ile Asp Glu Leu Pro Val Asp Phe Ala Ala Tyr Leu 390 395 400	1317
GGC GAG TGA GCGGGCCCCCT GCCCCCCTTC CCTGCCCCCC TCTCGCGACG Gly Glu	1366

85

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CCACTCTCTC GGTCGTCTGT CTCCCACGCG CGCACGCAGT TGATTTCGCG CTTCTTAAAC	60
GCTCTCTTTT TTTTATTTT TCACCTGACC AACCGCACCA CGTCGGCCTC CATC ATG	117
	Met
	1
TCG CAG CAA GAC CGA GTT GCC CCA CAG GAC CAG GAC TCG TTC CTC GAC	165
Ser Gln Gln Asp Arg Val Ala Pro Gln Asp Gln Asp Ser Phe Leu Asp	
5 10 15	
GAC CAG CCC GGC GTC CGC CCG ATC CCG TCC TTC GAT GAC ATG CCG TTG	213
Asp Gln Pro Gly Val Arg Pro Ile Pro Ser Phe Asp Asp Met Pro Leu	
20 25 30	
CAC CAG AAC CTT CTG CGC GGC ATC TAC TCG TAC GGC TTC GAG AAA CCG	261
His Gln Asn Leu Leu Arg Gly Ile Tyr Ser Tyr Gly Phe Glu Lys Pro	
35 40 45	
TCC AGC ATC CAG CAG CGC GCC ATC GCC CCC TTC ACG CGC GGC GGC GAC	309
Ser Ser Ile Gln Gln Arg Ala Ile Ala Pro Phe Thr Arg Gly Gly Asp	
50 55 60 65	
ATC ATC GCG CAG GCG CAG TCC GGT ACC GGC AAG ACG GGC GCC TTC TCC	357
Ile Ile Ala Gln Ala Gln Ser Gly Thr Gly Lys Thr Gly Ala Phe Ser	
70 75 80	
ATC GGC CTG CTG CAG CGC CTG GAC TTC CGC CAC AAC CTG ATC CAG GGC	405
Ile Gly Leu Leu Gln Arg Leu Asp Phe Arg His Asn Leu Ile Gln Gly	
85 90 95	
CTC GTG CTC TCC CCG ACC CGC GAG CTG GCC CTG CAG ACG GCG GAG GTG	453
Leu Val Leu Ser Pro Thr Arg Glu Leu Ala Leu Gln Thr Ala Glu Val	
100 105 110	
ATC AGC CGC ATC GGC GAG TTC CTG TCG AAC AGC GCG AAG TTC TGT GAG	501
Ile Ser Arg Ile Gly Glu Phe Leu Ser Asn Ser Ala Lys Phe Cys Glu	
115 120 125	
ACC TTT GTG GGT GGC ACG CGC GTG CAG GAT GAC CTG CGC AAG CTG CAG	549
Thr Phe Val Gly Gly Thr Arg Val Gln Asp Asp Leu Arg Lys Leu Gln	
130 135 140 145	
GCT GGC GTC GTC GTC GCC GTG GGG ACG CCG GGC CGC GTG TCC GAC GTG	597
Ala Gly Val Val Val Ala Val Gly Thr Pro Gly Arg Val Ser Asp Val	
150 155 160	
ATC AAG CGC GGC GCG CTG CGC ACC GAG TCC CTG CGC GTG CTG GTG CTC	645
Ile Lys Arg Gly Ala Leu Arg Thr Glu Ser Leu Arg Val Leu Val Leu	
165 170 175	
GAC GAG GCT GAT GAG ATG CTG TCT CAG GGC TTC GCG GAT CAG ATT TAC	693
Asp Glu Ala Asp Glu Met Leu Ser Gln Gly Phe Ala Asp Gln Ile Tyr	
180 185 190	

84

Asp Val Glu Lys Ser Ile Leu Ile Val Asp Gly Leu Tyr Arg Asp Gly
 370 375 380

Pro Ala Tyr Gln Thr Gly Ile Arg Leu Gly Asp Val Leu Leu Arg Ile
 385 390 395 400

Ala Gly Val Tyr Val Asp Ser Ile Ala Lys Ala Arg Gln Val Val Asp
 405 410 415

Ala Arg Cys Arg Cys Gly Cys Val Val Pro Val Thr Leu Ala Thr Lys
 420 425 430

Met Asn Gln Gln Tyr Ser Val Ala Leu Tyr Ile Met Thr Val Asp Pro
 435 440 445

Gln His Asn Asp Lys Pro Phe Phe Phe Asp Val His Ile His His Arg
 450 455 460

Ile Glu Ser Ser His Met Gly Lys Lys Ala Gln Trp Met Glu Val Leu
 465 470 475 480

Glu Ser Pro Ser Val Ser Ser Ala Ala Thr Thr Pro Leu Val Pro Leu
 485 490 495

Leu Arg Glu Pro Thr Pro Arg Arg Gly Ser Glu Leu Gln Ser Ser Ala
 500 505 510

Arg Ser Ala Phe Val Ala Thr Ser Tyr Phe Ser Ser Ala Arg Arg Ser
 515 520 525

Val Ser Ser Glu Ser Glu Arg Pro Arg Gly Ser Ser Ser Val Ala Met
 530 535 540

Ala Glu Glu Ala Ile Ala Leu Ala Pro Gln Gly Tyr Thr Pro Pro Asn
 545 550 555 560

Gln Val Arg Gly Arg Ser
 565

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1618 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 115..1323

Ala	Glu	Leu	Glu	Ala	Ala	Glu	Glu	Ala	Ala	Arg	Leu	Glu	Ala	Met	His	
				85					90					95		
Glu	Ala	Glu	Gln	Ala	Arg	Ser	Gln	Ala	Leu	Glu	Glu	Ala	Ala	Arg	Leu	
				100					105					110		
Arg	Ala	Glu	Leu	Glu	Glu	Ala	Glu	Glu	Ala	Ala	Arg	Leu	Asp	Val	Met	
				115					120					125		
His	Ala	Ala	Glu	Gln	Ala	Arg	Val	Gln	Ala	Leu	Glu	Glu	Ala	Ala	Arg	
				130					135					140		
Leu	Arg	Ala	Glu	Leu	Glu	Glu	Ala	Glu	Glu	Ala	Ala	Arg	Leu	Glu	Ala	
				145					150					155		
Met	His	Glu	Ala	Glu	Gln	Ala	Arg	Ser	Gln	Ala	Leu	Glu	Glu	Ala	Ala	
				165					170					175		
Arg	Leu	Arg	Ala	Glu	Leu	Glu	Ala	Ala	Glu	Glu	Ala	Ala	Arg	Leu	Asp	
				180					185					190		
Val	Met	His	Glu	Ala	Glu	Gln	Ala	Arg	Val	Gln	Ala	Leu	Glu	Glu	Ala	
				195					200					205		
Ala	Arg	Leu	Asp	Val	Met	His	Glu	Ala	Glu	Gln	Ala	Arg	Val	Gln	Ala	
				210					215					220		
Leu	Glu	Glu	Ala	Ala	Arg	Leu	Arg	Ala	Glu	Leu	Glu	Ala	Ala	Glu	Glu	
				225					230					235		
Ala	Ala	Arg	Leu	Asp	Val	Met	His	Glu	Ala	Glu	Gln	Ala	Arg	Val	Gln	
				245					250					255		
Ala	Leu	Glu	Glu	Ala	Ala	Arg	Leu	Arg	Ala	Glu	Leu	Glu	Ala	Ala	Glu	
				260					265					270		
Glu	Ala	Ala	Arg	Leu	Asp	Val	Met	His	Glu	Gly	Glu	Gln	Ala	Arg	Val	
				275					280					285		
Gln	Ala	Leu	Glu	Glu	Ala	Ala	Arg	Leu	Glu	Ala	Met	His	Glu	Ala	Glu	
				290					295					300		
Gln	Ala	Arg	Ser	Gln	Ala	Leu	Glu	Glu	Ala	Ala	Arg	Leu	Cys	Ala	Glu	
				305					310					315		
Leu	Glu	Ala	Glu	Glu	Glu	Glu	Lys	Asp	Glu	Arg	Pro	Ala	Thr	Ser	Ser	
				325					330					335		
Tyr	Ser	Glu	Glu	Cys	Lys	Gly	Arg	Leu	Leu	Ser	Arg	Ala	Arg	Pro	Asp	
				340					345					350		
Pro	Arg	Arg	Pro	Leu	Pro	Arg	Pro	Phe	Ile	Gly	Met	Ser	Leu	Leu	Glu	
				355					360					365		

82

Ile Glu Ser Ser His Met Gly Lys Lys Ala Gln Trp Met Glu Val Leu
 465 470 475 480

GAG AGC CCA TCC GTA TCT TCG GCT GCC ACC ACC CCT CTC GTG CCG CTC 1488
 Glu Ser Pro Ser Val Ser Ser Ala Ala Thr Thr Pro Leu Val Pro Leu
 485 490 495

TTG CGT GAG CCG ACG CCG CGT AGG GGC TCA GAG CTG CAG TCA AGT GCT 1536
 Leu Arg Glu Pro Thr Pro Arg Arg Gly Ser Glu Leu Gln Ser Ser Ala
 500 505 510

CGT TCC GCC TTC GTT GCC ACG TCT TAC TTC TCG AGC GCG CGC AGG TCG 1584
 Arg Ser Ala Phe Val Ala Thr Ser Tyr Phe Ser Ser Ala Arg Arg Ser
 515 520 525

GTC AGC TCA GAA AGT GAG CGA CCG CGC GGG TCC TCT AGC GTG GCT ATG 1632
 Val Ser Ser Glu Ser Glu Arg Pro Arg Gly Ser Ser Ser Val Ala Met
 530 535 540

GCG GAG GAG GCG ATC GCG CTG GCG CCG CAA GGG TAT ACC CCA CCC AAC 1680
 Ala Glu Glu Ala Ile Ala Leu Ala Pro Gln Gly Tyr Thr Pro Pro Asn
 545 550 555 560

CAA GTG CGC GGC CGT AGT TGACGTCTCT GTGTGAGTGT GTGTCGCTCC 1728
 Gln Val Arg Gly Arg Ser
 565

GTCTCCTTCC TTTTTCGTCA TGTGTTTTAT TCATTCTTTT TTC 1771

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 566 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Gln Ala Arg Val Gln Ala Leu Glu Glu Ala Ala Arg Leu Arg Ala Glu
 1 5 10 15

Leu Glu Ala Ala Glu Glu Ala Ala Arg Leu Asp Val Met His Ala Ala
 20 25 30

Glu Gln Ala Arg Val Gln Ala Leu Glu Glu Ala Ala Arg Leu Arg Ala
 35 40 45

Glu Leu Glu Glu Ala Glu Glu Ala Ala Arg Leu Asp Val Met His Ala
 50 55 60

Ala Glu Gln Ala Arg Val Gln Ala Leu Glu Glu Ala Ala Arg Leu Arg
 65 70 75 80

81

245																250						255						
GCC	CTC	GAG	GAG	GCA	GCG	CGT	CTC	CGC	GCG	GAG	CTG	GAG	GCG	GCC	GAG	816												
Ala	Leu	Glu	Glu	Ala	Ala	Arg	Leu	Arg	Ala	Glu	Leu	Glu	Ala	Ala	Glu													
260				265				270																				
GAG	GCG	GCC	CGC	CTG	GAT	GTC	ATG	CAC	GAG	GGC	GAG	CAG	GCC	CGT	GTC	864												
Glu	Ala	Ala	Arg	Leu	Asp	Val	Met	His	Glu	Gly	Glu	Gln	Ala	Arg	Val													
275				280				285																				
CAG	GCC	CTC	GAG	GAG	GCG	GCC	CGC	CTG	GAG	GCC	ATG	CAC	GAG	GCC	GAG	912												
Gln	Ala	Leu	Glu	Glu	Ala	Ala	Arg	Leu	Glu	Ala	Met	His	Glu	Ala	Glu													
290				295				300																				
CAG	GCC	CGC	TCC	CAG	GCC	CTC	GAG	GAG	GCA	GCG	CGT	CTC	TGC	GCG	GAG	960												
Gln	Ala	Arg	Ser	Gln	Ala	Leu	Glu	Glu	Ala	Ala	Arg	Leu	Cys	Ala	Glu													
305	310				315				320																			
CTG	GAG	GCT	GAG	GAG	GAG	GAA	AAA	GAT	GAG	CGG	CCG	GCG	ACG	TCG	AGC	1008												
Leu	Glu	Ala	Glu	Glu	Glu	Glu	Lys	Asp	Glu	Arg	Pro	Ala	Thr	Ser	Ser													
325				330				335																				
TAC	AGC	GAG	GAG	TGC	AAA	GGG	CGA	CTG	CTA	TCG	AGG	GCG	CGG	CCG	GAT	1056												
Tyr	Ser	Glu	Glu	Cys	Lys	Gly	Arg	Leu	Leu	Ser	Arg	Ala	Arg	Pro	Asp													
340				345				350																				
CCG	CGG	AGG	CCG	CTG	CCG	CGG	CCG	TTC	ATT	GGG	ATG	TCA	CTG	TTG	GAG	1104												
Pro	Arg	Arg	Pro	Leu	Pro	Arg	Pro	Phe	Ile	Gly	Met	Ser	Leu	Leu	Glu													
355				360				365																				
GAT	GTG	GAG	AAG	AGT	ATT	CTC	ATT	GTG	GAC	GGG	CTC	TAC	AGG	GAT	GGG	1152												
Asp	Val	Glu	Lys	Ser	Ile	Leu	Ile	Val	Asp	Gly	Leu	Tyr	Arg	Asp	Gly													
370				375				380																				
CCG	GCG	TAC	CAG	ACG	GGC	ATC	CGC	CTC	GGG	GAT	GTC	CTC	TTG	CGT	ATC	1200												
Pro	Ala	Tyr	Gln	Thr	Gly	Ile	Arg	Leu	Gly	Asp	Val	Leu	Leu	Arg	Ile													
385	390				395				400																			
GCG	GGG	GTT	TAC	GTG	GAT	TCA	ATA	GCG	AAG	GCG	AGG	CAG	GTG	GTC	GAT	1248												
Ala	Gly	Val	Tyr	Val	Asp	Ser	Ile	Ala	Lys	Ala	Arg	Gln	Val	Val	Asp													
405				410				415																				
GCG	CGT	TGC	CGC	TGC	GGC	TGC	GTC	GTT	CCC	GTG	ACG	CTG	GCG	ACG	AAG	1296												
Ala	Arg	Cys	Arg	Cys	Gly	Cys	Val	Val	Pro	Val	Thr	Leu	Ala	Thr	Lys													
420				425				430																				
ATG	AAC	CAG	CAG	TAC	AGC	GTG	GCT	CTG	TAT	ATC	ATG	ACG	GTG	GAT	CCG	1344												
Met	Asn	Gln	Gln	Tyr	Ser	Val	Ala	Leu	Tyr	Ile	Met	Thr	Val	Asp	Pro													
435				440				445																				
CAG	CAC	AAC	GAC	AAG	CCC	TTT	TTT	TTT	GAT	GTG	CAC	ATC	CAC	CAC	CGC	1392												
Gln	His	Asn	Asp	Lys	Pro	Phe	Phe	Phe	Asp	Val	His	Ile	His	His	Arg													
450				455				460																				
ATC	GAG	AGC	TCG	CAC	ATG	GGG	AAG	AAG	GCG	CAG	TGG	ATG	GAA	GTT	CTT	1440												

80

GAG CAG GCC CGT GTC CAG GCC CTC GAG GAG GCA GCG CGT CTC CGC GCG Glu Gln Ala Arg Val Gln Ala Leu Glu Glu Ala Ala Arg Leu Arg Ala 35 40 45	144
GAG CTG GAG GAG GCC GAG GAG GCG GCC CGC CTG GAT GTC ATG CAT GCG Glu Leu Glu Glu Ala Glu Glu Ala Ala Arg Leu Asp Val Met His Ala 50 55 60	192
GCC GAG CAG GCC CGC GTC CAG GCC CTC GAG GAG GCA GCG CGT CTC CGC Ala Glu Gln Ala Arg Val Gln Ala Leu Glu Glu Ala Ala Arg Leu Arg 65 70 75 80	240
GCG GAG CTG GAG GCT GCC GAG GAG GCG GCG CGC CTG GAG GCC ATG CAC Ala Glu Leu Glu Ala Ala Glu Glu Ala Ala Arg Leu Glu Ala Met His 85 90 95	288
GAG GCC GAG CAG GCC CGC TCC CAG GCC CTC GAG GAG GCA GCG CGT CTC Glu Ala Glu Gln Ala Arg Ser Gln Ala Leu Glu Glu Ala Ala Arg Leu 100 105 110	336
CGC GCG GAG CTG GAG GAA GCC GAG GAG GCG GCC CGC CTG GAT GTC ATG Arg Ala Glu Leu Glu Glu Ala Glu Glu Ala Ala Arg Leu Asp Val Met 115 120 125	384
CAT GCG GCC GAG CAG GCC CGC GTC CAG GCC CTC GAG GAG GCA GCG CGT His Ala Ala Glu Gln Ala Arg Val Gln Ala Leu Glu Glu Ala Ala Arg 130 135 140	432
CTC CGC GCG GAG CTG GAG GAG GCC GAG GAG GCG GCC CGC CTG GAG GCC Leu Arg Ala Glu Leu Glu Glu Ala Glu Glu Ala Ala Arg Leu Glu Ala 145 150 155 160	480
ATG CAC GAG GCC GAG CAG GCC CGC TCC CAG GCC CTC GAG GAG GCA GCG Met His Glu Ala Glu Gln Ala Arg Ser Gln Ala Leu Glu Glu Ala Ala 165 170 175	528
CGT CTC CGC GCG GAG CTG GAG GCG GCC GAG GAG GCG GCC CGC CTG GAT Arg Leu Arg Ala Glu Leu Glu Ala Ala Glu Glu Ala Ala Arg Leu Asp 180 185 190	576
GTC ATG CAC GAG GCC GAG CAG GCC CGT GTC CAG GCC CTC GAG GAG GCG Val Met His Glu Ala Glu Gln Ala Arg Val Gln Ala Leu Glu Glu Ala 195 200 205	624
GCG CGC CTG GAT GTC ATG CAC GAG GCC GAG CAG GCC CGC GTC CAG GCC Ala Arg Leu Asp Val Met His Glu Ala Glu Gln Ala Arg Val Gln Ala 210 215 220	672
CTC GAG GAG GCA GCG CGT CTC CGC GCG GAG CTG GAG GCG GCC GAG GAG Leu Glu Glu Ala Ala Arg Leu Arg Ala Glu Leu Glu Ala Ala Glu Glu 225 230 235 240	720
GCG GCC CGC CTG GAT GTC ATG CAC GAG GCC GAG CAG GCC CGC GTC CAG Ala Ala Arg Leu Asp Val Met His Glu Ala Glu Gln Ala Arg Val Gln 768	

79

Lys Leu Cys Lys Thr Met Lys Glu Val Leu Gly Asp Lys Val Glu Lys
500 505 510

Val Thr Val Ser Glu Arg Leu Leu Thr Ser Pro Cys Ile Leu Val Thr
515 520 525

Ser Glu Phe Gly Trp Ser Ala His Met Glu Gln Ile Met Arg Asn Gln
530 535 540

Ala Leu Arg Asp Ser Ser Met Ala Gln Tyr Met Val Ser Lys Lys Thr
545 550 555 560

Met Glu Val Asn Pro Asp His Pro Ile Ile Lys Glu Leu Arg Arg Arg
565 570 575

Val Glu Ala Asp Glu Asn Asp Lys Ala Val Lys Asp Leu Val Phe Leu
580 585 590

Leu Phe Asp Thr Ser Leu Leu Thr Ser Gly Phe Gln Leu Asp Asp Pro
595 600 605

Thr Gly Tyr Ala Glu Arg Ile Asn Arg Met Ile Lys Leu Gly Leu Ser
610 615 620

Leu Asp Glu Glu Glu Glu Glu Val Ala Glu Ala Pro Pro Ala Glu Ala
625 630 635 640

Ala Pro Ala Glu Val Thr Ala Gly Thr Ser Ser Met Glu Gln Val Asp
645 650 655

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1771 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..1698

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CAG GCC CGC GTC CAG GCC CTC GAG GAG GCA GCG CGT CTC CGC GCG GAG 48
Gln Ala Arg Val Gln Ala Leu Glu Glu Ala Ala Arg Leu Arg Ala Glu
1 5 10 15

CTG GAG GCG GCC GAG GAG GCG GCC CGC CTG GAT GTC ATG CAT GCG GCC 96
Leu Glu Ala Ala Glu Glu Ala Ala Arg Leu Asp Val Met His Ala Ala
20 25 30

78

195	200	205
Glu Val Lys Lys Thr Tyr Glu Val Lys Asn Lys His Lys Pro Leu Trp		
210	215	220
Thr Arg Asp Thr Lys Asp Val Thr Lys Glu Glu Tyr Ala Ala Phe Tyr		
225	230	235 240
Lys Ala Ile Ser Asn Asp Trp Glu Asp Thr Ala Ala Thr Lys His Phe		
	245	250 255
Ser Val Glu Gly Gln Leu Glu Phe Arg Ala Ile Ala Phe Val Pro Lys		
	260	265 270
Arg Ala Pro Phe Asp Met Phe Glu Pro Asn Lys Lys Arg Asn Asn Ile		
	275	280 285
Lys Leu Tyr Val Arg Arg Val Phe Ile Met Asp Asn Cys Glu Asp Leu		
	290	295 300
Cys Pro Asp Trp Leu Gly Phe Val Lys Gly Val Val Asp Ser Glu Asp		
305	310	315 320
Leu Pro Leu Asn Ile Ser Arg Glu Asn Leu Gln Gln Asn Lys Ile Leu		
	325	330 335
Lys Val Ile Arg Lys Asn Ile Val Lys Lys Cys Leu Glu Leu Phe Glu		
	340	345 350
Glu Ile Ala Glu Asn Lys Glu Asp Tyr Lys Gln Phe Tyr Glu Gln Phe		
	355	360 365
Gly Lys Asn Ile Lys Leu Gly Ile His Glu Asp Thr Ala Asn Arg Lys		
	370	375 380
Lys Leu Met Glu Leu Leu Arg Phe Tyr Ser Thr Glu Ser Gly Glu Glu		
385	390	395 400
Met Thr Thr Leu Lys Asp Tyr Val Thr Arg Met Lys Pro Glu Gln Lys		
	405	410 415
Ser Ile Tyr Tyr Ile Thr Gly Asp Ser Lys Lys Lys Leu Glu Ser Ser		
	420	425 430
Pro Phe Ile Glu Lys Ala Arg Arg Cys Gly Leu Glu Val Leu Phe Met		
	435	440 445
Thr Glu Pro Ile Asp Glu Tyr Val Met Gln Gln Val Lys Asp Phe Glu		
	450	455 460
Asp Lys Lys Phe Ala Cys Leu Thr Lys Glu Gly Val His Phe Glu Glu		
465	470	475 480
Ser Glu Glu Glu Lys Lys Gln Arg Glu Glu Lys Lys Ala Ala Cys Glu		
	485	490 495

77

815

820

825

830

GAC TGAGCCGGTA A

2040

Asp

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 656 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```

Ser Leu Thr Asp Pro Ala Val Leu Gly Glu Glu Thr His Leu Arg Val
 1             5             10             15

Arg Val Val Pro Asp Lys Ala Asn Lys Thr Leu Thr Val Glu Asp Asn
      20             25             30

Gly Ile Gly Met Thr Lys Ala Asp Leu Val Asn Asn Leu Gly Thr Ile
      35             40             45

Ala Arg Ser Gly Thr Lys Ala Phe Met Glu Ala Leu Glu Ala Gly Gly
      50             55             60

Asp Met Ser Met Ile Gly Gln Phe Gly Val Gly Phe Tyr Ser Ala Tyr
      65             70             75             80

Leu Val Ala Asp Arg Val Thr Val Val Ser Lys Asn Asn Ser Asp Glu
      85             90             95

Ala Tyr Trp Glu Ser Ser Ala Gly Gly Thr Phe Thr Ile Thr Ser Val
      100            105            110

Gln Glu Ser Asp Met Lys Arg Gly Thr Ser Thr Thr Leu His Leu Lys
      115            120            125

Glu Asp Gln Gln Glu Tyr Leu Glu Glu Arg Arg Val Lys Glu Leu Ile
      130            135            140

Lys Lys His Ser Glu Phe Ile Gly Tyr Asp Ile Glu Leu Met Val Glu
      145            150            155            160

Lys Thr Ala Glu Lys Glu Val Thr Asp Glu Asp Glu Glu Glu Asp Glu
      165            170            175

Ser Lys Lys Lys Ser Cys Gly Asp Glu Gly Glu Pro Lys Val Glu Glu
      180            185            190

Val Thr Glu Gly Gly Glu Asp Lys Lys Lys Lys Thr Lys Lys Val Lys

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TCG	CCG	TTC	ATC	GAG	AAG	GCG	AGA	CGC	TGC	GGG	CTC	GAG	GTG	CTG	TTC		1402
Ser	Pro	Phe	Ile	Glu	Lys	Ala	Arg	Arg	Cys	Gly	Leu	Glu	Val	Leu	Phe		
			610					615					620				
ATG	ACG	GAG	CCG	ATC	GAC	GAG	TAC	GTG	ATG	CAG	CAG	GTG	AAG	GAC	TTC		1450
Met	Thr	Glu	Pro	Ile	Asp	Glu	Tyr	Val	Met	Gln	Gln	Val	Lys	Asp	Phe		
			625				630					635					
GAG	GAC	AAG	AAG	TTC	GCG	TGC	CTG	ACG	AAG	GAA	GGC	GTG	CAC	TTC	GAG		1498
Glu	Asp	Lys	Lys	Phe	Ala	Cys	Leu	Thr	Lys	Glu	Gly	Val	His	Phe	Glu		
			640			645					650						
GAG	TCC	GAG	GAG	GAG	AAG	AAG	CAG	CGC	GAG	GAG	AAG	AAG	GCG	GCG	TGC		1546
Glu	Ser	Glu	Glu	Glu	Lys	Lys	Gln	Arg	Glu	Glu	Lys	Lys	Ala	Ala	Cys		
					660					665					670		
GAG	AAG	CTG	TGC	AAG	ACG	ATG	AAG	GAG	GTG	CTG	GGC	GAC	AAG	GTG	GAG		1594
Glu	Lys	Leu	Cys	Lys	Thr	Met	Lys	Glu	Val	Leu	Gly	Asp	Lys	Val	Glu		
				675				680						685			
AAG	GTG	ACC	GTG	TCG	GAG	CGC	CTG	TTG	ACG	TCG	CCG	TGC	ATC	CTG	GTG		1642
Lys	Val	Thr	Val	Ser	Glu	Arg	Leu	Leu	Thr	Ser	Pro	Cys	Ile	Leu	Val		
			690					695					700				
ACG	TCG	GAG	TTT	GGG	TGG	TCG	GCG	CAC	ATG	GAA	CAG	ATC	ATG	CGC	AAC		1690
Thr	Ser	Glu	Phe	Gly	Trp	Ser	Ala	His	Met	Glu	Gln	Ile	Met	Arg	Asn		
			705				710					715					
CAG	GCG	CTG	CGC	GAC	TCC	AGC	ATG	GCG	CAG	TAC	ATG	GTG	TCC	AAG	AAG		1738
Gln	Ala	Leu	Arg	Asp	Ser	Ser	Met	Ala	Gln	Tyr	Met	Val	Ser	Lys	Lys		
			720			725					730						
ACG	ATG	GAG	GTG	AAC	CCC	GAC	CAC	CCC	ATC	ATC	AAG	GAG	CTG	CGC	CGC		1786
Thr	Met	Glu	Val	Asn	Pro	Asp	His	Pro	Ile	Ile	Lys	Glu	Leu	Arg	Arg		
					740				745						750		
CGC	GTG	GAG	GCG	GAC	GAG	AAC	GAC	AAG	GCC	GTG	AAG	GAC	CTC	GTC	TTC		1834
Arg	Val	Glu	Ala	Asp	Glu	Asn	Asp	Lys	Ala	Val	Lys	Asp	Leu	Val	Phe		
				755				760						765			
CTG	CTC	TTC	GAC	ACG	TCG	CTG	CTC	ACG	TCC	GGC	TTC	CAG	CTG	GAT	GAC		1882
Leu	Leu	Phe	Asp	Thr	Ser	Leu	Leu	Thr	Ser	Gly	Phe	Gln	Leu	Asp	Asp		
			770					775					780				
CCC	ACC	GGC	TAC	GCC	GAG	CGC	ATC	AAC	CGC	ATG	ATC	AAG	CTC	GGC	CTG		1930
Pro	Thr	Gly	Tyr	Ala	Glu	Arg	Ile	Asn	Arg	Met	Ile	Lys	Leu	Gly	Leu		
			785				790					795					
TCG	CTC	GAC	GAG	GAG	GAG	GAG	GAG	GTC	GCC	GAG	GCG	CCG	CCG	GCC	GAG		1978
Ser	Leu	Asp	Glu	Glu	Glu	Glu	Glu	Val	Ala	Glu	Ala	Pro	Pro	Ala			

75

AAG GAG GTG AAG AAG ACG TAC GAG GTC AAG AAC AAG CAC AAG CCG CTC Lys Glu Val Lys Lys Thr Tyr Glu Val Lys Asn Lys His Lys Pro Leu 385 390 395	730
TGG ACG CGC GAC ACG AAG GAC GTG ACG AAG GAG GAG TAC GCG GCC TTC Trp Thr Arg Asp Thr Lys Asp Val Thr Lys Glu Glu Tyr Ala Ala Phe 400 405 410	778
TAC AAG GCC ATC TCC AAC GAC TGG GAG GAC ACG GCG GCG ACG AAG CAC Tyr Lys Ala Ile Ser Asn Asp Trp Glu Asp Thr Ala Ala Thr Lys His 415 420 425 430	826
TTC TCG GTG GAG GGC CAG CTG GAG TTC CGC GCG ATC GCG TTC GTG CCG Phe Ser Val Glu Gly Gln Leu Glu Phe Arg Ala Ile Ala Phe Val Pro 435 440 445	874
AAG CGC GCG CCG TTC GAC ATG TTC GAG CCG AAC AAG AAG CGC AAC AAC Lys Arg Ala Pro Phe Asp Met Phe Glu Pro Asn Lys Lys Arg Asn Asn 450 455 460	922
ATC AAG CTG TAC GTG CGC CGC GTG TTC ATC ATG GAC AAC TGC GAG GAC Ile Lys Leu Tyr Val Arg Arg Val Phe Ile Met Asp Asn Cys Glu Asp 465 470 475	970
CTG TGC CCG GAC TGG CTC GGC TTC GTG AAG GGC GTC GTG GAC AGC GAG Leu Cys Pro Asp Trp Leu Gly Phe Val Lys Gly Val Val Asp Ser Glu 480 485 490	1018
GAC CTG CCG CTG AAC ATC TCG CGC GAG AAC CTG CAG CAG AAC AAG ATC Asp Leu Pro Leu Asn Ile Ser Arg Glu Asn Leu Gln Gln Asn Lys Ile 495 500 505 510	1066
CTG AAG GTG ATC CGC AAG AAC ATC GTG AAG AAG TGC CTG GAG CTG TTC Leu Lys Val Ile Arg Lys Asn Ile Val Lys Lys Cys Leu Glu Leu Phe 515 520 525	1114
GAA GAG ATA GCG GAG AAC AAG GAG GAC TAC AAG CAG TTC TAC GAG CAG Glu Glu Ile Ala Glu Asn Lys Glu Asp Tyr Lys Gln Phe Tyr Glu Gln 530 535 540	1162
TTC GGC AAG AAC ATC AAG CTG GGC ATC CAC GAG GAC ACG GCG AAC CGC Phe Gly Lys Asn Ile Lys Leu Gly Ile His Glu Asp Thr Ala Asn Arg 545 550 555	1210
AAG AAG CTG ATG GAG TTG CTG CGC TTC TAC AGC ACC GAG TCG GGG GAG Lys Lys Leu Met Glu Leu Leu Arg Phe Tyr Ser Thr Glu Ser Gly Glu 560 565 570	1258
GAG ATG ACG ACA CTG AAG GAC TAC GTG ACG CGC ATG AAG CCG GAG CAG Glu Met Thr Thr Leu Lys Asp Tyr Val Thr Arg Met Lys Pro Glu Gln 575 580 585 590	1306
AAG TCG ATC TAC TAC ATC ACT GGC GAC AGC AAG AAG AAG CTG GAG TCG Lys Ser Ile Tyr Tyr Ile Thr Gly Asp Ser Lys Lys Lys Leu Glu Ser 595 600 605	1354

CGCGGTGGCG GCCGCTCTAG AACTAGTGGG TCCCCCGGGC TGCAGGAATT CGGCACGAGA	60
G AGC CTG ACG GAC CCG GCG GTG CTG GGC GAG GAG ACT CAC CTG CGC	106
Ser Leu Thr Asp Pro Ala Val Leu Gly Glu Glu Thr His Leu Arg	
180 185 190	
GTC CGC GTG GTG CCG GAC AAG GCG AAC AAG ACG CTG ACG GTG GAG GAT	154
Val Arg Val Val Pro Asp Lys Ala Asn Lys Thr Leu Thr Val Glu Asp	
195 200 205	
AAC GGC ATC GGC ATG ACC AAG GCG GAC CTC GTG AAC AAT CTG GGC ACG	202
Asn Gly Ile Gly Met Thr Lys Ala Asp Leu Val Asn Asn Leu Gly Thr	
210 215 220	
ATC GCG CGC TCC GGC ACG AAG GCT TTC ATG GAG GCA CTG GAG GCC GGC	250
Ile Ala Arg Ser Gly Thr Lys Ala Phe Met Glu Ala Leu Glu Ala Gly	
225 230 235	
GGC GAC ATG AGC ATG ATC GGC CAG TTC GGT GTC GGC TTC TAC TCC GCG	298
Gly Asp Met Ser Met Ile Gly Gln Phe Gly Val Gly Phe Tyr Ser Ala	
240 245 250	
TAC CTT GTG GCG GAC CGC GTG ACG GTG GTG TCG AAG AAC AAC TCG GAC	346
Tyr Leu Val Ala Asp Arg Val Thr Val Val Ser Lys Asn Asn Ser Asp	
255 260 265 270	
GAG GCG TAC TGG GAA TCG TCT GCG GGG GGC ACG TTC ACC ATC ACG AGC	394
Glu Ala Tyr Trp Glu Ser Ser Ala Gly Gly Thr Phe Thr Ile Thr Ser	
275 280 285	
GTG CAG GAG TCG GAC ATG AAG CGC GGC ACG AGT ACA ACG CTG CAC CTA	442
Val Gln Glu Ser Asp Met Lys Arg Gly Thr Ser Thr Thr Leu His Leu	
290 295 300	
AAG GAG GAC CAG CAG GAG TAC CTG GAG GAG CGC CGG GTG AAG GAG CTG	490
Lys Glu Asp Gln Gln Glu Tyr Leu Glu Glu Arg Arg Val Lys Glu Leu	
305 310 315	
ATC AAG AAG CAC TCC GAG TTC ATC GGC TAC GAC ATC GAG CTG ATG GTG	538
Ile Lys Lys His Ser Glu Phe Ile Gly Tyr Asp Ile Glu Leu Met Val	
320 325 330	
GAG AAG ACG GCG GAG AAG GAG GTG ACG GAC GAG GAC GAG GAG GAG GAC	586
Glu Lys Thr Ala Glu Lys Glu Val Thr Asp Glu Asp Glu Glu Glu Asp	
335 340 345 350	
GAG TCG AAG AAG AAG TCC TGC GGG GAC GAG GGC GAG CCG AAG GTG GAG	634
Glu Ser Lys Lys Lys Ser Cys Gly Asp Glu Gly Glu Pro Lys Val Glu	
355 360 365	
GAG GTG ACG GAG GGC GGC GAG GAC AAG AAG AAG AAG ACG AAG AAG GTG	682
Glu Val Thr Glu Gly Gly Glu Asp Lys Lys Lys Lys Thr Lys Lys Val	
370 375 380	

73

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```

Met Val Lys Ser His Tyr Ile Cys Ala Gly Arg Leu Val Arg Ile Leu
 1             5             10             15

Arg Gly Pro Arg Gln Asp Arg Val Gly Val Ile Val Asp Ile Val Asp
      20             25             30

Ala Asn Arg Val Leu Val Glu Asn Pro Glu Asp Ala Lys Met Trp Arg
      35             40             45

His Val Gln Asn Leu Lys Asn Val Glu Pro Leu Lys Tyr Cys Val Ser
 50             55             60

Val Ser Arg Asn Cys Ser Ala Lys Ala Leu Lys Asp Ala Leu Ala Ser
 65             70             75             80

Ser Lys Ala Leu Glu Lys Tyr Ala Lys Thr Arg Thr Ala Ala Arg Val
      85             90             95

Glu Ala Lys Lys Ala Cys Ala Ala Ser Thr Asp Phe Glu Arg Tyr Gln
     100             105             110

Leu Arg Val Ala Arg Arg Ser Arg Ala His Trp Ala Arg Lys Val Phe
     115             120             125

Asp Glu Lys Asp Ala Lys Thr Pro Val Ser Trp His Lys Val Ala Leu
     130             135             140

Lys Lys Met Gln Lys Lys Ala Ala Lys Met Asp Ser Thr Glu Gly Ala
     145             150             155             160

Lys Arg Arg Met Gln Lys Ala Ile Ala Ala Arg Lys Ala Lys Lys
     165             170             175

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(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2040 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 62..2029

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

72

GGC CGC CTG GTG CGC ATC CTG CGT GGC CCC CGC CAG GAC CGC GTT GGT Gly Arg Leu Val Arg Ile Leu Arg Gly Pro Arg Gln Asp Arg Val Gly 560 565 570	100
GTG ATC GTC GAC ATT GTC GAC GCG AAC CGC GTG CTG GTG GAG AAC CCG Val Ile Val Asp Ile Val Asp Ala Asn Arg Val Leu Val Glu Asn Pro 575 580 585	148
GAG GAC GCG AAG ATG TGG CGC CAC GTG CAG AAC CTG AAG AAC GTG GAG Glu Asp Ala Lys Met Trp Arg His Val Gln Asn Leu Lys Asn Val Glu 590 595 600	196
CCG CTG AAG TAC TGC GTG AGC GTC AGC CGC AAC TGC AGC GCG AAG GCG Pro Leu Lys Tyr Cys Val Ser Val Ser Arg Asn Cys Ser Ala Lys Ala 605 610 615	244
CTG AAG GAT GCG CTG GCC TCG TCG AAG GCG CTG GAG AAG TAC GCG AAG Leu Lys Asp Ala Leu Ala Ser Ser Lys Ala Leu Glu Lys Tyr Ala Lys 620 625 630 635	292
ACG CGC ACT GCT GCG CGC GTG GAG GCG AAG AAG GCG TGC GCC GCG TCG Thr Arg Thr Ala Ala Arg Val Glu Ala Lys Lys Ala Cys Ala Ala Ser 640 645 650	340
ACG GAC TTC GAG CGC TAC CAG CTG CGC GTT GCG CGC CGT TCT CGC GCG Thr Asp Phe Glu Arg Tyr Gln Leu Arg Val Ala Arg Arg Ser Arg Ala 655 660 665	388
CAC TGG GCG CGC AAG GTG TTC GAC GAG AAG GAC GCG AAG ACG CCC GTG His Trp Ala Arg Lys Val Phe Asp Glu Lys Asp Ala Lys Thr Pro Val 670 675 680	436
TCG TGG CAC AAG GTT GCG CTG AAG AAG ATG CAG AAG AAG GCC GCA AAG Ser Trp His Lys Val Ala Leu Lys Lys Met Gln Lys Lys Ala Ala Lys 685 690 695	484
ATG GAC TCG ACC GAG GGC GCT AAG AGG CGC ATG CAG AAG GCG ATC GCT Met Asp Ser Thr Glu Gly Ala Lys Arg Arg Met Gln Lys Ala Ile Ala 700 705 710 715	532
GCC CGC AAG GCG AAA AAG TAAGGCCATA CCCTCACTTC GCTTGTTTCG Ala Arg Lys Ala Lys Lys 720	580
TGATTTTTTCG TGGGAGTCGG TGGCCCTACC AGCGGTCTTT CATTGGCTTA TTTCTATCCG	640
GTCTGAAAGA GGTACAAAAA AAAAAAAAAA AAAAAA	676

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 175 amino acids

(B) TYPE: amino acid

71

370

375

380

Ile Lys Arg Asn Pro Ala Glu His Thr Ser Tyr Ser Asn Arg Ala Ala
 385 390 395 400

Ala Tyr Ile Lys Leu Gly Ala Phe Asn Asp Ala Leu Lys Asp Ala Glu
 405 410 415

Lys Cys Ile Glu Leu Lys Pro Asp Phe Val Lys Gly Tyr Ala Arg Lys
 420 425 430

Gly His Ala Tyr Phe Trp Thr Lys Gln Tyr Asn Arg Ala Leu Gln Ala
 435 440 445

Tyr Asp Glu Gly Leu Lys Val Asp Pro Ser Asn Ala Asp Cys Lys Asp
 450 455 460

Gly Arg Tyr Arg Thr Ile Met Lys Ile Gln Glu Met Ala Ser Gly Gln
 465 470 475 480

Ser Ala Asp Gly Asp Glu Ala Ala Arg Arg Ala Met Asp Asp Pro Glu
 485 490 495

Ile Ala Ala Ile Met Gln Asp Ser Tyr Met Gln Leu Val Leu Lys Glu
 500 505 510

Met Gln Asn Asp Pro Thr Arg Ile Gln Glu Tyr Met Lys Asp Ser Gly
 515 520 525

Ile Ser Ser Lys Ile Asn Lys Leu Ile Ser Ala Gly Ile Ile Arg Phe
 530 535 540

Gly Gln
 545

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 676 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 26..550

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

AATTCGGCAC GAGGCATTGT GCATA ATG GTC AAG TCC CAC TAC ATC TGC GCG
 Met Val Lys Ser His Tyr Ile Cys Ala
 550 555

52

70

Leu His Gly Met Arg Arg Tyr Asp Asp Ala Ile Ala Ala Tyr Glu Lys
 85 90 95

Gly Leu Lys Val Asp Pro Ser Asn Ser Gly Cys Ala Gln Gly Val Lys
 100 105 110

Asp Val Gln Val Ala Lys Ala Arg Glu Ala Arg Asp Pro Ile Ala Arg
 115 120 125

Val Phe Thr Pro Glu Ala Phe Arg Lys Ile Gln Glu Asn Pro Lys Leu
 130 135 140

Ser Leu Leu Met Leu Gln Pro Asp Tyr Val Lys Met Val Asp Thr Val
 145 150 155 160

Ile Arg Asp Pro Ser Gln Gly Arg Leu Tyr Met Glu Asp Gln Arg Phe
 165 170 175

Ala Leu Thr Leu Met Tyr Leu Ser Gly Met Lys Ile Pro Asn Asp Gly
 180 185 190

Asp Gly Glu Glu Glu Glu Arg Pro Ser Ala Lys Ala Ala Glu Thr Ala
 195 200 205

Lys Pro Lys Glu Glu Lys Pro Leu Thr Asp Asn Glu Lys Glu Ala Leu
 210 215 220

Ala Leu Lys Glu Glu Gly Asn Lys Leu Tyr Leu Ser Lys Lys Phe Glu
 225 230 235 240

Glu Ala Leu Thr Lys Tyr Gln Glu Ala Gln Val Lys Asp Pro Asn Asn
 245 250 255

Thr Leu Tyr Ile Leu Asn Val Ser Ala Val Tyr Phe Glu Gln Gly Asp
 260 265 270

Tyr Asp Lys Cys Ile Ala Glu Cys Glu His Gly Ile Glu His Gly Arg
 275 280 285

Glu Asn His Cys Asp Tyr Thr Ile Ile Ala Lys Leu Met Thr Arg Asn
 290 295 300

Ala Leu Cys Leu Gln Arg Gln Arg Lys Tyr Glu Ala Ala Ile Asp Leu
 305 310 315 320

Tyr Lys Arg Ala Leu Val Glu Trp Arg Asn Pro Asp Thr Leu Lys Lys
 325 330 335

Leu Thr Glu Cys Glu Lys Glu His Gln Lys Ala Val Glu Glu Ala Tyr
 340 345 350

Ile Asp Pro Glu Ile Ala Lys Gln Lys Lys Asp Glu Gly Asn Gln Tyr
 355 360 365

Phe Lys Glu Asp Lys Phe Pro Glu Ala Val Ala Ala Tyr Thr Glu Ala

69

TGCAACGATT TCGTTTTATT TACGTCTGTG TAGCTCCTCT ATTCAACGGT GCGATGACGC	2408
TAACGAAGCT GGCCTGTATT CGGCTAAGGC GAAGGCAAAA GACTAGGAGG GGGGGGGGAA	2468
GGAGACGGCG TGACCATCAC TGCGAAGAAA CAAGCCGAAG AAAAGGCCCC GAACGCCTGC	2528
ATTTCCGCGC GCCCTCGCCC GCCTTCCTTC CTTCTTCGC TCTCTCTCTC TCTCTCTCTC	2588
GCTATCTTCT CAACGGAGAC ATGAAAGGCG TTTGTTAGGA AAAGAGGGGG GGGGGAAGAG	2648
TGGGACGACG CGCTGCGTCT TTTGGGCACT GGTCACGTGC GTCACCCTCT TTTTTATCT	2708
CTATTGGCAC TGTCTTGTTT CTTTCCCTT TCCTATCATA CGCGTCTCGC AAACGACTCC	2768
GCGCTGAGCA GCCATGTGCT GCGGCGTGGA GGAAGTACAC AGACATCACG GATGCATATG	2828
TGCGCGTCCG TGTACGCGCT TGTATGGGGC TTCTAACAGC GCCTGTGTGT GTTTGTGTGT	2888
GTGTGTGTGT GTGTGTCTGT GTATTTTCGAG CGTCTGTATG CTATTCTATT AAGCACCGAA	2948
GAAGAGACAC ACACGACAGC GAAGGAGATG GTGTCGGCTT TTCGGCTAAT CACTCCCTTC	3008
CATAGCTTCT CTGAAGGAGG CTCTCTTCCA GAGGAATAGA CTGCAGATGG GGTCCACGTT	3068
TATCTGAGGA GTCAACGGAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA	3128
CTCGAG	3134

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 546 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met	Asp	Ala	Thr	Glu	Leu	Lys	Asn	Lys	Gly	Asn	Glu	Glu	Phe	Ser	Ala	1	5	10	15
Gly	Arg	Tyr	Val	Glu	Ala	Val	Asn	Tyr	Phe	Ser	Lys	Ala	Ile	Gln	Leu	20	25	30	
Asp	Glu	Gln	Asn	Ser	Val	Leu	Tyr	Ser	Asn	Arg	Ser	Ala	Cys	Phe	Ala	35	40	45	
Ala	Met	Gln	Lys	Tyr	Lys	Asp	Ala	Leu	Asp	Asp	Ala	Asp	Lys	Cys	Ile	50	55	60	
Ser	Ile	Lys	Pro	Asn	Trp	Ala	Lys	Gly	Tyr	Val	Arg	Arg	Gly	Ala	Ala	65	70	75	80

68

Phe	Lys	Glu	Asp	Lys	Phe	Pro	Glu	Ala	Val	Ala	Tyr	Thr	Glu	Ala		
370						375					380					
ATC AAG CGC AAC CCT GCC GAG CAC ACC TCC TAC AGC AAT CGC GCG GCC	1620															
Ile Lys Arg Asn Pro Ala Glu His Thr Ser Tyr Ser Asn Arg Ala Ala																
385	390	395	400													
GCG TAC ATC AAG CTT GGA GCC TTC AAC GAC GCC CTC AAG GAC GCG GAG	1668															
Ala Tyr Ile Lys Leu Gly Ala Phe Asn Asp Ala Leu Lys Asp Ala Glu																
	405	410	415													
AAG TGC ATT GAG CTG AAG CCC GAC TTT GTT AAG GGC TAC GCG CGC AAG	1716															
Lys Cys Ile Glu Leu Lys Pro Asp Phe Val Lys Gly Tyr Ala Arg Lys																
	420	425	430													
GGT CAT GCT TAC TTT TGG ACC AAG CAG TAC AAC CGC GCG CTG CAG GCG	1764															
Gly His Ala Tyr Phe Trp Thr Lys Gln Tyr Asn Arg Ala Leu Gln Ala																
	435	440	445													
TAC GAT GAG GGC CTC AAG GTG GAC CCG AGC AAT GCG GAC TGC AAG GAT	1812															
Tyr Asp Glu Gly Leu Lys Val Asp Pro Ser Asn Ala Asp Cys Lys Asp																
	450	455	460													
GGG CGG TAT CGC ACA ATC ATG AAG ATT CAG GAG ATG GCA TCT GGC CAA	1860															
Gly Arg Tyr Arg Thr Ile Met Lys Ile Gln Glu Met Ala Ser Gly Gln																
465	470	475	480													
TCC GCG GAT GGC GAC GAG GCG GCG CGC CGG GCC ATG GAC GAT CCT GAA	1908															
Ser Ala Asp Gly Asp Glu Ala Ala Arg Arg Ala Met Asp Asp Pro Glu																
	485	490	495													
ATC GCG GCA ATC ATG CAA GAT AGC TAC ATG CAA CTA GTG TTG AAG GAG	1956															
Ile Ala Ala Ile Met Gln Asp Ser Tyr Met Gln Leu Val Leu Lys Glu																
	500	505	510													
ATG CAG AAC GAT CCC ACG CGC ATT CAG GAG TAC ATG AAG GAC TCC GGG	2004															
Met Gln Asn Asp Pro Thr Arg Ile Gln Glu Tyr Met Lys Asp Ser Gly																
	515	520	525													
ATC TCA TCG AAG ATC AAC AAG CTG ATT TCA GCT GGC ATC ATT CGT TTT	2052															
Ile Ser Ser Lys Ile Asn Lys Leu Ile Ser Ala Gly Ile Ile Arg Phe																
530	535	540														
GGT CAG TAGACTTCTA CGCTGCCTCA TCTTTTCCGT GTCTTTGCGT CGGCGGGTAT	2108															
Gly Gln																
545																
CGTAAAGCAC AATAAAGCAG CGATTACAT GCACGAGTAA AGTGCTGCGC CTCTCAAACA	2168															
CGACGTCGAG GCTGTGGTGC AGATGCGCGT CCTGCATGAA GGTAGTGAAG AGGAAAGTAA	2228															
GGGATGTTGT TTGTGGGCCT TCGTGGCTGC GCACACACCT CTTATCTCCT TCGCTTGGTA	2288															
CCTTCTCCCT TTTTCGTCTT CACCCCCCTT TCTCTTCTCA CGCTCTCCCT GGCGCGGTGG	2348															

67

145	150	155	160	
ATC CGC GAC CCT TCG CAG GGC CGG CTG TAC ATG GAA GAC CAG CGC TTT				948
Ile Arg Asp Pro Ser Gln Gly Arg Leu Tyr Met Glu Asp Gln Arg Phe				
165		170	175	
GCC CTG ACG CTC ATG TAC CTG AGC GGA ATG AAG ATT CCC AAC GAT GGT				996
Ala Leu Thr Leu Met Tyr Leu Ser Gly Met Lys Ile Pro Asn Asp Gly				
180		185	190	
GAT GGC GAG GAG GAG GAA CGT CCG TCT GCG AAG GCG GCA GAG ACA GCG				1044
Asp Gly Glu Glu Glu Glu Arg Pro Ser Ala Lys Ala Ala Glu Thr Ala				
195		200	205	
AAG CCA AAA GAG GAG AAG CCT CTC ACC GAC AAC GAG AAG GAG GCC CTG				1092
Lys Pro Lys Glu Glu Lys Pro Leu Thr Asp Asn Glu Lys Glu Ala Leu				
210		215	220	
GCG CTC AAG GAG GAG GGC AAC AAG CTG TAC CTC TCG AAG AAG TTT GAG				1140
Ala Leu Lys Glu Glu Gly Asn Lys Leu Tyr Leu Ser Lys Lys Phe Glu				
225		230	235	240
GAG GCG CTG ACC AAG TAC CAA GAG GCG CAG GTG AAA GAC CCC AAC AAC				1188
Glu Ala Leu Thr Lys Tyr Gln Glu Ala Gln Val Lys Asp Pro Asn Asn				
245		250	255	
ACT TTA TAC ATT CTG AAC GTG TCG GCC GTG TAC TTC GAG CAG GGT GAC				1236
Thr Leu Tyr Ile Leu Asn Val Ser Ala Val Tyr Phe Glu Gln Gly Asp				
260		265	270	
TAC GAC AAG TGC ATC GCC GAG TGC GAG CAC GGT ATC GAG CAC GGT CGC				1284
Tyr Asp Lys Cys Ile Ala Glu Cys Glu His Gly Ile Glu His Gly Arg				
275		280	285	
GAG AAC CAC TGC GAC TAC ACA ATC ATT GCG AAG CTC ATG ACC CGG AAC				1332
Glu Asn His Cys Asp Tyr Thr Ile Ile Ala Lys Leu Met Thr Arg Asn				
290		295	300	
GCC TTG TGC CTC CAG AGG CAG AGG AAG TAC GAG GCT GCT ATC GAC CTT				1380
Ala Leu Cys Leu Gln Arg Gln Arg Lys Tyr Glu Ala Ala Ile Asp Leu				
305		310	315	320
TAC AAG CGC GCC CTT GTC GAG TGG CGT AAC CCT GAC ACC CTC AAG AAG				1428
Tyr Lys Arg Ala Leu Val Glu Trp Arg Asn Pro Asp Thr Leu Lys Lys				
325		330	335	
CTG ACG GAG TGC GAG AAG GAG CAC CAA AAG GCG GTG GAG GAA GCC TAC				1476
Leu Thr Glu Cys Glu Lys Glu His Gln Lys Ala Val Glu Glu Ala Tyr				
340		345	350	
ATC GAT CCT GAG ATC GCG AAG CAG AAG AAA GAC GAA GGT AAC CAG TAC				1524
Ile Asp Pro Glu Ile Ala Lys Gln Lys Lys Asp Glu Gly Asn Gln Tyr				
355		360	365	
TTC AAG GAG GAT AAG TTC CCC GAG GCC GTG GCA GCG TAC ACG GAG GCC				1572

(xi) SEQUENCE DESCRIPTION: SkeQ ID NO:1:

CAAGTGTCGA AGGACAGTGT TCNCCGTGTG AGATCGCCGG CTGTGCGTGT GAAGGCGGTG	60
CCATCGGANA AACAAACACCG GTGGANCCGC AGGAAACCAT CTTTCTCCGC AGGTCTCTTT	120
TTGTTGTCGA TTGAGAGTGC NCCAAACCTT GCTGGTGCCC TTCTCACATA TCATGTTTTT	180
CGTTGTGCGC TCGCTTTGCC TTTCTCTCC TTTCCCTCTC TTCCGTGGTG CCGTGATAC	240
TTCTGGCACC CGCTACGTCA CTTGCTGGT TTGAACAGAA CCACTGTGAA CACCCACGGG	300
CGATCGCACA CATAACATC CCTCACTCAC ACACACAGCT ACATCTATCC TACATAAAGC	360
TGAAAAAAAA GTCTACGAAC AATTTTGT TTACAGTGCG TTGCCGCACA TTTCTCCGTA	420
ATG GAC GCA ACT GAG CTG AAG AAC AAG GGG AAC GAA GAG TTC TCC GCC	468
Met Asp Ala Thr Glu Leu Lys Asn Lys Gly Asn Glu Glu Phe Ser Ala	
1 5 10 15	
GGC CGC TAT GTG GAG GCG GTG AAC TAC TTC TCA AAG GCG ATC CAG TTG	516
Gly Arg Tyr Val Glu Ala Val Asn Tyr Phe Ser Lys Ala Ile Gln Leu	
20 25 30	
GAT GAG CAG AAC AGT GTC CTC TAC AGC AAC CGC TCC GCC TGT TTT GCA	564
Asp Glu Gln Asn Ser Val Leu Tyr Ser Asn Arg Ser Ala Cys Phe Ala	
35 40 45	
GCC ATG CAG AAA TAC AAG GAC GCG CTG GAC GAC GCC GAC AAG TGC ATC	612
Ala Met Gln Lys Tyr Lys Asp Ala Leu Asp Asp Ala Asp Lys Cys Ile	
50 55 60	
TCG ATC AAG CCG AAT TGG GCC AAG GGC TAC GTG CGC CGA GGA GCA GCT	660
Ser Ile Lys Pro Asn Trp Ala Lys Gly Tyr Val Arg Arg Gly Ala Ala	
65 70 75 80	
CTC CAT GGC ATG CGC CGC TAC GAC GAT GCC ATT GCC GCG TAT GAA AAG	708
Leu His Gly Met Arg Arg Tyr Asp Asp Ala Ile Ala Ala Tyr Glu Lys	
85 90 95	
GGG CTC AAG GTG GAC CCT TCC AAC AGC GGC TGC GCG CAG GGC GTG AAG	756
Gly Leu Lys Val Asp Pro Ser Asn Ser Gly Cys Ala Gln Gly Val Lys	
100 105 110	
GAC GTG CAG GTA GCC AAG GCC CGC GAA GCA CGT GAC CCC ATC GCT CGC	804
Asp Val Gln Val Ala Lys Ala Arg Glu Ala Arg Asp Pro Ile Ala Arg	
115 120 125	
GTC TTC ACC CCG GAG GCG TTC CGC AAG ATC CAA GAG AAT CCC AAG CTG	852
Val Phe Thr Pro Glu Ala Phe Arg Lys Ile Gln Glu Asn Pro Lys Leu	
130 135 140	
TCT CTA CTT ATG CTG CAG CCG GAC TAC GTG AAG ATG GTA GAC ACC GTC	900
Ser Leu Leu Met Leu Gln Pro Asp Tyr Val Lys Met Val Asp Thr Val	

65

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Corixa Corporation
- (ii) TITLE OF INVENTION: *LEISHMANIA* ANTIGENS FOR USEIN THE THERAPY AND
DIAGNOSIS OF LEISHMANIASIS
- (iii) NUMBER OF SEQUENCES: 87
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: SEED and BERRY LLP
 - (B) STREET: 6300 Columbia Center, 701 Fifth Avenue
 - (C) CITY: Seattle
 - (D) STATE: Washington
 - (E) COUNTRY: USA
 - (F) ZIP: 98104-7092
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: US
 - (B) FILING DATE: 12-FEB-1998
 - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Maki, David J.
 - (B) REGISTRATION NUMBER: 31,392
 - (C) REFERENCE/DOCKET NUMBER: 210121.42001PC
- (ix) TELECOMMUNICATION INFORMATION:
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(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 3134 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 421..2058

EXAMPLE 15

SYNTHESIS OF POLYPEPTIDES

Polypeptides may be synthesized on a Perkin Elmer/Applied Biosystems
5 Division 430A peptide synthesizer using Fmoc chemistry with HPTU (O-Benzotriazole-N,N,N',N'-tetramethyluronium hexafluorophosphate) activation. A Gly-Cys-Gly sequence may be attached to the amino terminus of the peptide to provide a method of conjugation, binding to an immobilized surface, or labeling of the peptide. Cleavage of the peptides from the solid support may be carried out using the following
10 cleavage mixture: trifluoroacetic acid:ethanedithiol:thioanisole:water:phenol (40:1:2:2:3). After cleaving for 2 hours, the peptides may be precipitated in cold methyl-t-butyl-ether. The peptide pellets may then be dissolved in water containing 0.1% trifluoroacetic acid (TFA) and lyophilized prior to purification by C18 reverse phase HPLC. A gradient of 0%-60% acetonitrile (containing 0.1% TFA) in water
15 (containing 0.1% TFA) may be used to elute the peptides. Following lyophilization of the pure fractions, the peptides may be characterized using electrospray or other types of mass spectrometry and by amino acid analysis.

20 From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for the purpose of illustration, various modifications may be made without deviating from the spirit and scope of the invention.

EXAMPLE 15ISOLATION OF DNA ENCODING FOR *L. MAJOR* ANTIGENS
BY CD4+ T CELL EXPRESSION CLONING

5

This example illustrates the isolation of T cell antigens of *L. major* using a direct T cell screening approach.

Leishmania-specific CD4+ T cell lines were derived from the PBMC of an individual who tested positive in a leishmania skin test but had no clinical history of
10 disease. These T cell lines were used to screen a *L. major* amastigote cDNA expression library prepared as described in Example 1. Immunoreactive clones were isolated and sequenced as described above. The determined cDNA sequences for the 8 isolated clones referred to as 1G6-34, 1E6-44, 4A5-63, 1B11-39, 2A10-37, 4G2-83, 4H6-41, 8G3-100 are provided in SEQ ID NO: 72-79, respectively, with the corresponding
15 predicted amino acid sequences being provided in SEQ ID NO: 80-87, respectively. The cDNA sequences provided for 1E6-44, 2A10-37, 4G2-83, 4H6-41 and 8G3-100 are believed to represent partial clones. All of these clones were shown to stimulate T cell proliferation.

Comparison of these sequences with those in the gene bank as described
20 above revealed no known homologies to the antigen 4A5-63. 1G6-34 was found to have some homology to histone H2B previously identified in *L. enrietti*. Antigens 1E6-44, 1B11-39 and 8G3-100 showed some homology to sequences previously identified in other eukaryotes, in particular *Saccharomyces cerevisiae*. 2A10-37 and 4H6-41 were found to be homologous to the two previously identified proteins alpha tubulin from *L.*
25 *donovani* and beta tubulin from *L. major*, respectively, and 4G2-83 was found to be homologous to elongation initiation factor 2 previously identified in *T. cruzi*.

EXAMPLE 14ISOLATION AND CHARACTERIZATION OF DNA ENCODING FOR SOLUBLE ANTIGENS FROM
AN *L. CHAGASI* GENOMIC DNA LIBRARY

5 This example illustrates the preparation of five soluble *Leishmania* antigen genes from an *L. chagasi* genomic DNA library.

 An *L. chagasi* genomic DNA expression library was prepared from *L. chagasi* promastigotes using the unidirectional Lambda ZAP (uni-ZAP) kit (Stratagene) according to the manufacturer's protocol. This library was screened with a high titer
10 rabbit sera raised against *L. major* soluble antigens, as described above in Example 9. Five positive clones were identified. The phagemid were excised and DNA from each of the Five clones was sequenced using a Perkin Elmer/Applied Biosystems Division automated sequencer Model 373A. The DNA sequences for these antigens, referred to as LcgSP1, LcgSP3, LcgSP4, LcgSP8, and LcgSP10 are provided in SEQ ID NO:44-
15 48, respectively, with the corresponding amino acid sequences being provided in SEQ ID NO:49-53, respectively.

 Comparison of these sequences with known sequences in the gene bank as described above, revealed no known homologies to LcgSP3, LcgSP4, LcgSP8 and LcgSP10. LcgSP1 was found to be homologous to the known antigen HSP70.

20 Figures 30A and B illustrate the proliferative response of murine lymph nodes to recombinant LcgSP8, LcgSP10 and LcgSP3. Lymph nodes were taken BALB/c mice 17 days after infection with *L. major*. Infection occurred by footpad injection of 2×10^6 parasites/footpad. The cells were stimulated with recombinant antigen and proliferation was measured at 72 hours using ^3H -thymidine. Figure 30A
25 shows the CPM, a direct measurement of mitotic activity in response to the antigens, and Figure 30B shows the stimulation index, which measures the proliferative response relative to the negative control.

In order to obtain a higher specificity for the detection of antibodies in sera from visceral leishmaniasis patients, a homologue of LmgSP9 was isolated from *L. chagasi*, one of the causative agents of visceral leishmaniasis. A total of 80,000 pfu of an amplified *L. chagasi* genomic library were screened with the entire coding region of LmgSP9 (amplified from *L. major* genomic DNA). Seven hybridizing clones were purified to homogeneity. The determined DNA sequences for two of these clones, referred to as Lc Gene A and LcGene B, are provided in SEQ ID NO: 59 and 60, respectively, with the corresponding predicted amino acid sequences being provided in SEQ ID NO: 61 and 62, respectively. The open reading frame for Lc Gene A was found to show some homology to Gene A/C, previously isolated from *L. major* (McKlean et al., *Mol. Bio. Parasitol.*, 85:221-231, 1997). The open reading frame for Lc Gene B showed some homology to Gene B of *L. major*, discussed above, and was found to contain eleven repeats of a 14 amino acid repeat unit (SEQ ID NO: 63), with each repeat being further divided into two 7 amino acid units, provided in SEQ ID NO: 64 and 65.

The diagnostic potentials of Lc Gene A and Lc Gene B were evaluated by ELISA as described above using sera from visceral leishmaniasis patients from Sudan and Brazil, and from uninfected controls. Absorbance values were compared to those obtained using LmgSP9. Much higher absorbance values were obtained with Lc Gene A and Lc Gene B than with LmgSP9, with Lc Gene B appearing to be more effective than Lc Gene A in detecting antibodies in certain cases. These results indicate that Lc Gene B is highly effective in the diagnosis of visceral leishmaniasis.

In order to assess the diagnostic potential of the repeats found within Lc Gene B, a series of 6 peptides were synthesized (SEQ ID NO: 66-71; referred to as Pep 1-6), differing in an R or H residue. An ELISA was carried out using the full-length LcGene B protein and the six peptides. The absorbance values obtained with Pep 3 were higher than those obtained with the other 5 peptides, however they were not as high as those obtained with the full length protein.

Table 2

REACTIVITY OF LMGSP9 WITH SERA FROM LEISHMANIA PATIENTS

Patient No.	<i>L. chagasi</i> lysate	K39	LmgSP9
Sudanese samples:			
B19	1.067	0.306	0.554
B25	1.884	3.435	0.974
B43	1.19	3.225	0.86
B47	2.405	2.892	0.375
B50	0.834	0.748	0.432
B58	0.921	0.235	0.92
B63	1.291	0.303	0.764
B70	0.317	0.089	3.056
VL4	1.384	3.035	2.965
VL11	0.382	0.144	0.142
VL12	0.277	0.068	0.098
VL13	0.284	0.12	0.194
Brazilian samples:			
105	3.508	3.53	0.374
106	2.979	3.373	2.292
107	2.535	3.444	0.46
109	1.661	3.415	3.319
111	3.595	3.537	0.781
112	2.052	3.469	0.63
113	3.352	3.429	0.963
114	2.316	3.437	1.058
115	2.073	3.502	1.186
116	3.331	3.461	0.96
Normal Donors:			
129	0.157	0.104	0.08
130	0.195	0.076	0.095
131	0.254	0.134	0.086
132	0.102	0.035	0.043

succinyl-6-hydroxy-2,4-cyclohexadiene-1-carboxylic acid synthase). LmgSP9 and LmgSP19 were found to be homologous to a *L. major* hydrophilic surface protein referred to as Gene B (Flinn, H.M. et al. *Mol. Biochem. Parasit.* 65:259-270, 1994), and to ubiquitin, respectively. To the best of the inventors' knowledge, none of these
5 antigens have been previously shown to elicit T or B cell responses.

The reactivity of recombinant LmgSP9 with sera from patients with visceral leishmaniasis, (from both Sudan and Brazil) and from normal donors was evaluated by ELISA as described above. The absorbance values were compared with those obtained using the known *Leishmania* antigen K39 described above, with *L.*
10 *chagasi* lysate being employed as a positive control. Representative results of these assays are provided below in Table 2, wherein all the patients from Brazil and those from the Sudan designated as "VL" were inflicted with visceral leishmaniasis. The results demonstrated that LmgSP9 specifically detects antibody in most individuals with visceral leishmaniasis, regardless of geographical location. In several cases, the
15 absorbance values of the antibody reactivity to LmgSP9 were comparable to that observed with K39. In addition, LmgSP9 detected several cases of leishmaniasis that were not detected using K39. These results indicate that LmgSP9 can be used to complement the reactivity of K39.

alone. These results demonstrate that the *Leishmania* antigens of the present invention are effective in conferring protection against *Leishmania* infection.

EXAMPLE 13

5 ISOLATION OF DNA ENCODING FOR SOLUBLE ANTIGENS FROM AN *L. MAJOR* GENOMIC DNA LIBRARY

This example illustrates the isolation of seven soluble *Leishmania* antigen genes from an *L. major* genomic DNA library.

10 An *L. major* genomic DNA expression library was prepared from *L. major* promastigotes using the unidirectional Lambda ZAP (uni-ZAP) kit (Stratagene) according to the manufacturer's protocol. This library was screened with a high titer rabbit sera raised against *L. major* soluble antigens, as described above in Example 9. Seven positive clones were identified. The phagemid were excised and DNA from
15 each of the seven clones was sequenced using a Perkin Elmer/Applied Biosystems Division automated sequencer Model 373A. The DNA sequences for these antigens, referred to as LmgSP1, LmgSP3, LmgSP5, LmgSP8, LmgSP9, LmgSP13, LmgSP19, are provided in SEQ ID NO:29-35, respectively, with the corresponding amino acid sequences being provided in SEQ ID NO: 36-42, respectively. LmgSP13 was found to
20 contain a 39 amino acid repeat sequence shown in SEQ ID NO:43.

Subsequent studies resulted in the isolation of a full-length sequence for LmgSP9. The full-length DNA sequence is provided in SEQ ID NO: 54, with the corresponding predicted amino acid sequence being provided in SEQ ID NO: 55. The amino acid sequence was found to contain six 14 amino acid repeat units (SEQ ID NO:
25 56), with each unit being further divided into two 7 amino acid units, provided in SEQ ID NO: 57 and 58.

Comparison of the DNA and amino acid sequences for the isolated antigens as described above, revealed no significant homologies to LmgSP1, LmgSP3, and LmgSP13. LmgSP5 was found to be related to the known PSA2 family. LmgSP8
30 was found to bear some homology to a sequence previously identified in *E. coli* (2-

dilutions of sera from BALB/c mice previously administered either (i) saline solution; (ii) the adjuvant *B. pertussis*; (iii) soluble *Leishmania* antigens plus *B. pertussis*; (iv) live *L. major* promastigotes; or (v) soluble *Leishmania* antigens plus *B. pertussis* followed by live *L. major* promastigotes (as described below in Example 12).

- 5 Considerably higher absorbances were seen with sera from mice infected with live *L. major* promastigotes and with mice infected with live *L. major* promastigotes following immunization with soluble *Leishmania* antigens plus *B. pertussis*, than with sera from the other three groups of mice, indicating that anti-MAPS-1A antibody titers increase following *Leishmania* infection.

10

EXAMPLE 12

USE OF *LEISHMANIA* ANTIGENS FOR VACCINATION AGAINST *LEISHMANIA* INFECTION

- 15 This example illustrates the effectiveness of *Leishmania* antigens in conferring protection against disease in the experimental murine leishmaniasis model system. For a discussion of the murine leishmaniasis model system see, for example, Reiner *et al. Annu. Rev. Immunol.*, 13:151-77, 1995.

- The effectiveness of (i) crude soluble *Leishmania* antigens, (ii) MAPS-
20 1A, and (iii) a mixture of Ldp23, LbeiF4A and M15, as vaccines against *Leishmania* infection was determined as follows. BALB/c mice (5 per group) were immunized intra-peritoneally three times at biweekly intervals with either (i) 30 µg crude soluble *Leishmania* antigens, (ii) 20 µg MAPS-1A or (iii) a mixture containing 10 µg each of LeIF, Ldp23 and M15, together with 100 µg of the adjuvant *C. parvum*. Two control
25 groups were immunized with either saline or *C. parvum* alone. Two weeks after the last immunization, the mice were challenged with 2×10^5 late-log phase promastigotes of *L. major*. Infection was monitored weekly by measurement of footpad swelling. The amount of footpad swelling seen in mice immunized with either crude soluble *Leishmania* antigens, a mixture of Ldp23, LbeiF4A and M15 (Figure 28), or MAPS-1A
30 (Figure 29) was significantly less than that seen in mice immunized with *C. parvum*

infection were highly responsive to MAPS-1A, whereas cells isolated 10 days post-infection were unresponsive.

EXAMPLE 11

5 IMMUNOREACTIVITY OF SOLUBLE *LEISHMANIA* ANTIGENS WITH SERA FROM *LEISHMANIA*-INFECTED PATIENTS

The reactivity of MAPS-1A with sera from uninfected individuals, from human leishmaniasis patients with cutaneous infection, from human patients with acute
10 visceral leishmaniasis, and from *L. major*-infected BALB/c mice was determined as follows.

Assays were performed in 96-well plates coated with 200 ng antigen diluted to 50 μ L in carbonate coating buffer, pH 9.6. The wells were coated overnight at 4 °C (or 2 hours at 37 °C). The plate contents were then removed and the wells were
15 blocked for 2 hours with 200 μ L of PBS/1% BSA. After the blocking step, the wells were washed five times with PBS/0.1% Tween 20TM. 50 μ L sera, diluted 1:100 in PBS/0.1% Tween 20TM/0.1% BSA, was then added to each well and incubated for 30 minutes at room temperature. The plates were then washed again five times with PBS/0.1% Tween 20TM.

20 The enzyme conjugate (horseradish peroxidase - Protein A, Zymed, San Francisco, CA) was then diluted 1:10,000 in PBS/0.1% Tween 20TM/0.1% BSA, and 50 μ L of the diluted conjugate was added to each well and incubated for 30 minutes at room temperature. Following incubation, the wells were washed five times with PBS/0.1% Tween 20TM. 100 μ L of tetramethylbenzidine peroxidase (TMB) substrate
25 (Kirkegaard and Perry Laboratories, Gaithersburg, MD) was added, undiluted, and incubated for about 15 minutes. The reaction was stopped with the addition of 100 μ L of 1 N H₂SO₄ to each well, and the plates were read at 450 nm.

As shown in Figure 26, approximately 50% of the samples from human leishmaniasis patients showed reactivities with recombinant MAPS-1A substantially
30 above background. Figure 27 shows the reactivity of MAPS-1A with increasing

Recombinant MAPS-1A protein having an amino-terminal HIS-Tag was prepared using a high level *E. coli* expression system and recombinant protein was purified by affinity chromatography as described in Example 1. Southern blot analysis of genomic DNA from *L. major* digested with a panel of restriction enzymes, seven
5 other *Leishmania* species digested with PstI, and two other infectious-disease pathogens (*T. cruzi* and *T. brucei*), using the full length insert of MAPS-1A, demonstrated that MAPS-1A is present in all eight *Leishmania* species tested (Figure 23). Northern blot analysis of *L. major* promastigote and amastigote RNAs indicated that MAPS-1A is constitutively expressed.

10 Using oligonucleotide primers (SEQ ID NOs:27 and 28) based on the MAPS-1A cDNA sequence provided in SEQ ID NO: 23, the corresponding gene was isolated from *L. tropica* by means of PCR (using 30 cycles of the following temperature step sequence: 94 °C, 1 minute; 50 °C, 1 minute; 72 °C, 1 minute) The determined cDNA sequence for the *L. tropica* MAPS-1A protein is provided in SEQ ID
15 NO: 25, with the corresponding amino acid sequence being provided in SEQ ID NO: 26.

The ability of recombinant MAPS-1A to stimulate cell proliferation was investigated as follows. PBMC from 3 *L. braziliensis*-infected patients having active mucosal leishmaniasis, from 4 patients post kala-azar infection (previously infected
20 with *L. chagasi* and/or *L. donovani*) and from 3 uninfected-individuals were prepared as described above in Example 7. The ability of MAPS-1A to stimulate proliferation of these PBMC was determined as described in Example 8 above. As shown in Figure 24, significant levels of MAPS-1A specific PBMC proliferation were seen in 2 of the 7 *Leishmania* patients.

25 The ability of MAPS-1A to stimulate proliferation in mice lymph node cultures was determined as described in Example 8. Figure 25 shows the amount of proliferation stimulated by MAPS-1A (at 25 µg/ml, 5 µg/ml and 1 µg/ml) as compared to that stimulated by the positive control ConA and by crude *L. major* promastigote supernatant proteins, 20 days post-infection with *L. major*. Cells isolated 20 days post-

Leishmania antigens or antigenic epitopes. Antigenic epitopes and polypeptides comprising such epitopes may be prepared by synthetic means, as described generally above and in detail in Example 15.

In certain aspects of the present invention, described in detail below, the polypeptides, antigenic epitopes and/or soluble *Leishmania* antigens may be incorporated into pharmaceutical compositions or vaccines. For clarity, the term "polypeptide" will be used when describing specific embodiments of the inventive therapeutic compositions and diagnostic methods. However, it will be clear to one of skill in the art that the antigenic epitopes of the present invention may also be employed in such compositions and methods.

Pharmaceutical compositions comprise one or more polypeptides, each of which may contain one or more of the above sequences (or variants thereof), and a physiologically acceptable carrier. Vaccines comprise one or more of the above polypeptides and a non-specific immune response enhancer, such as an adjuvant (e.g., LbIF4A, interleukin-12 or other cytokines) or a liposome (into which the polypeptide is incorporated). Vaccines may additionally contain a delivery vehicle, such as a biodegradable microsphere (disclosed, for example, in U.S. Patent Nos. 4,897,268 and 5,075,109). Pharmaceutical compositions and vaccines within the scope of the present invention may also contain other *Leishmania* antigens, either incorporated into a combination polypeptide or present within one or more separate polypeptides.

Alternatively, a pharmaceutical composition or vaccine may contain DNA encoding one or more of the polypeptides as described above, such that the polypeptide is generated *in situ*. In such pharmaceutical compositions and vaccines, the DNA may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid expression systems, bacteria and viral expression systems. Appropriate nucleic acid expression systems contain the necessary DNA sequences for expression in the patient (such as a suitable promoter and terminating signal). Bacterial delivery systems involve the administration of a bacterium (such as *Bacillus-Calmette-Guerrin*) that expresses an immunogenic portion of the polypeptide on its cell surface. In a preferred embodiment, the DNA may be

introduced using a viral expression system (e.g., vaccinia or other pox virus, retrovirus, or adenovirus), which may involve the use of a non-pathogenic (defective), replication competent virus. Techniques for incorporating DNA into such expression systems are well known to those of ordinary skill in the art. The DNA may also be "naked," as
5 described, for example, in Ulmer et al., *Science* 259:1745-1749 (1993) and reviewed by Cohen, *Science* 259:1691-1692 (1993). The uptake of naked DNA may be increased by coating the DNA onto biodegradable beads, which are efficiently transported into the cells.

While any suitable carrier known to those of ordinary skill in the art may
10 be employed in the pharmaceutical compositions of this invention, the type of carrier will vary depending on the mode of administration. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a fat, a wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum,
15 cellulose, glucose, sucrose, and magnesium carbonate, may be employed. Biodegradable microspheres (e.g., polylactic galactide) may also be employed as carriers for the pharmaceutical compositions of this invention. Suitable biodegradable microspheres are disclosed, for example, in U.S. Patent Nos. 4,897,268 and 5,075,109.

Any of a variety of adjuvants may be employed in the vaccines of this
20 invention to nonspecifically enhance the immune response. Most adjuvants contain a substance designed to protect the antigen from rapid catabolism, such as aluminum hydroxide or mineral oil, and a nonspecific stimulator of immune responses, such as lipid A, *Bordella pertussis* or *Mycobacterium tuberculosis*. Suitable adjuvants are commercially available as, for example, Freund's Incomplete Adjuvant and Complete
25 Adjuvant (Difco Laboratories, Detroit, MI), Merck Adjuvant 65 (Merck and Company, Inc., Rahway, NJ), alum, biodegradable microspheres, monophosphoryl lipid A and quil A. Preferred adjuvants include LbeIF4A, IL-12 and other cytokines such as IFN- γ or granulocyte-macrophage colony stimulating factor (GM-CSF). By virtue of its ability to induce an exclusive Th1 immune response, the use of LbeIF4A, and variants thereof,
30 as an adjuvant in the vaccines of the present invention is particularly preferred.

In one preferred embodiment, compositions of the present invention include multiple polypeptides selected so as to provide enhanced protection against a variety of *Leishmania* species. Such polypeptides may be selected based on the species of origin of the native antigen or based on a high degree of conservation of amino acid sequence among different species of *Leishmania*. A combination of individual polypeptides may be particularly effective as a prophylactic and/or therapeutic vaccine because (1) stimulation of proliferation and/or cytokine production by individual polypeptides may be additive, (2) stimulation of proliferation and/or cytokine production by individual polypeptides may be synergistic, (3) individual polypeptides may stimulate cytokine profiles in such a way as to be complementary to each other and/or (4) individual polypeptides may be complementary to one another when certain of them are expressed more abundantly on the individual species or strain of *Leishmania* responsible for infection. A preferred combination contains polypeptides that comprise immunogenic portions of M15, Ldp23, Lbhsp83, Lt-1 and LbeIF4A. Alternatively, or in addition, the combination may include one or more polypeptides comprising immunogenic portions of other *Leishmania* antigens disclosed herein, and/or soluble *Leishmania* antigens.

The above pharmaceutical compositions and vaccines may be used, for example, to induce protective immunity against *Leishmania* in a patient, such as a human or a dog, to prevent leishmaniasis. Appropriate doses and methods of administration for this purposes are described in detail below.

The pharmaceutical compositions and vaccines described herein may also be used to stimulate an immune response, which may be cellular and/or humoral, in a patient. For *Leishmania*-infected patients, the immune responses that may be generated include a preferential Th1 immune response (*i.e.*, a response characterized by the production of the cytokines interleukin-1, interleukin-2, interleukin-12 and/or interferon- γ , as well as tumor necrosis factor- α). For uninfected patients, the immune response may be the production of interleukin-12 and/or interleukin-2, or the stimulation of gamma delta T-cells. In either category of patient, the response stimulated may include IL-12 production. Such responses may also be elicited in

biological samples of PBMC or components thereof derived from *Leishmania*-infected or uninfected individuals. As noted above, assays for any of the above cytokines may generally be performed using methods known to those of ordinary skill in the art, such as an enzyme-linked immunosorbent assay (ELISA).

5 Suitable pharmaceutical compositions and vaccines for use in this aspect of the present invention are those that contain at least one polypeptide comprising an immunogenic portion of a *Leishmania* antigen disclosed herein (or a variant thereof). Preferably, the polypeptides employed in the pharmaceutical compositions and vaccines are complementary, as described above. Soluble *Leishmania* antigens, with or without
10 additional polypeptides, may also be employed.

 The pharmaceutical compositions and vaccines described herein may also be used to treat a patient afflicted with a disease responsive to IL-12 stimulation. The patient may be any warm-blooded animal, such as a human or a dog. Such diseases include infections (which may be, for example, bacterial, viral or protozoan) or diseases
15 such as cancer. In one embodiment, the disease is leishmaniasis, and the patient may display clinical symptoms or may be asymptomatic. In general, the responsiveness of a particular disease to IL-12 stimulation may be determined by evaluating the effect of treatment with a pharmaceutical composition or vaccine of the present invention on clinical correlates of immunity. For example, if treatment results in a heightened Th1
20 response or the conversion of a Th2 to a Th1 profile, with accompanying clinical improvement in the treated patient, the disease is responsive to IL-12 stimulation. Polypeptide administration may be as described below, or may extend for a longer period of time, depending on the indication. Preferably, the polypeptides employed in the pharmaceutical compositions and vaccines are complementary, as described above.
25 A particularly preferred combination contains polypeptides that comprise immunogenic portions of M15, Ldp23, Lbhsp83, Lt-1 and LbeIF4A, Lmsp1a, Lmsp9a, and MAPS-1A. Soluble *Leishmania* antigens, with or without additional polypeptides, may also be employed.

 Routes and frequency of administration, as well as dosage, for the above
30 aspects of the present invention will vary from individual to individual and may parallel

those currently being used in immunization against other infections, including protozoan, viral and bacterial infections. In general, the pharmaceutical compositions and vaccines may be administered by injection (*e.g.*, intracutaneous, intramuscular, intravenous or subcutaneous), intranasally (*e.g.*, by aspiration) or orally. Between 1 and 5 12 doses may be administered over a 1 year period. For therapeutic vaccination (*i.e.*, treatment of an infected individual), 12 doses are preferably administered, at one month intervals. For prophylactic use, 3 doses are preferably administered, at 3 month intervals. In either case, booster vaccinations may be given periodically thereafter. Alternate protocols may be appropriate for individual patients. A suitable dose is an 10 amount of polypeptide or DNA that, when administered as described above, is capable of raising an immune response in an immunized patient sufficient to protect the patient from leishmaniasis for at least 1-2 years. In general, the amount of polypeptide present in a dose (or produced *in situ* by the DNA in a dose) ranges from about 100 ng to about 1mg per kg of host, typically from about 10 μ g to about 100 μ g. Suitable dose sizes 15 will vary with the size of the patient, but will typically range from about 0.1 mL to about 5 mL.

In another aspect, this invention provides methods for using one or more of the polypeptides described above to diagnose *Leishmania* infection in a patient using a skin test. As used herein, a "skin test" is any assay performed directly on a patient in 20 which a delayed-type hypersensitivity (DTH) reaction (such as induration and accompanying redness) is measured following intradermal injection of one or more polypeptides as described above. Such injection may be achieved using any suitable device sufficient to contact the polypeptide or polypeptides with dermal cells of the patient, such as a tuberculin syringe or 1 mL syringe. Preferably, the reaction is 25 measured at least 48 hours after injection, more preferably 72 hours after injection.

The DTH reaction is a cell-mediated immune response, which is greater in patients that have been exposed previously to a test antigen (*i.e.*, an immunogenic portion of a polypeptide employed, or a variant thereof). The response may be measured visually, using a ruler. In general, induration that is greater than about 0.5 cm in 30 diameter, preferably greater than about 1.0 cm in diameter, is a positive response,

indicative of *Leishmania* infection, which may or may not be manifested as an active disease.

The polypeptides of this invention are preferably formulated, for use in a skin test, as pharmaceutical compositions containing at least one polypeptide and a physiologically acceptable carrier, as described above. Such compositions typically contain one or more of the above polypeptides in an amount ranging from about 1 μ g to 100 μ g, preferably from about 10 μ g to 50 μ g in a volume of 0.1 mL. Preferably, the carrier employed in such pharmaceutical compositions is a saline solution with appropriate preservatives, such as phenol and/or Tween 80™.

The inventive polypeptides may also be employed in combination with one or more known *Leishmania* antigens in the diagnosis of leishmaniasis, using, for example, the skin test described above. Preferably, individual polypeptides are chosen in such a way as to be complementary to each other. Examples of known *Leishmania* antigens which may be usefully employed in conjunction with the inventive polypeptides include K39 (Burns et al., Proc. Natl. Acad. Sci. USA, 1993 90:775-779).

The following Examples are offered by way of illustration and not by way of limitation.

EXAMPLES

EXAMPLE 1

PREPARATION OF M15

This Example illustrates the preparation of a *Leishmania* antigen M15, having the sequence provided in SEQ ID NO:2.

An *L. major* (Friedlan strain) amastigote cDNA expression library prepared in the λ ZAP II vector (Stratagene, La Jolla, CA) was screened according to manufacturer's instructions using sera obtained from *L. major* infected BALB/c mice (8 weeks post inoculation). Approximately 40,000 plaques were screened and four clones expressing reactive antigens were purified to homogeneity by two subsequent rounds of low density screening. Bluescript phagemid inserts were excised from positive clones

for further analysis. An *EcoRI/SstII* restriction fragment from the 5' end of one partial cDNA insert isolated during first round screening (pLma1-1) was subsequently used as a probe to rescreen for clones containing full length cDNA inserts. The probe was labeled to high specific activity (10^9 cpm/ μ g) with [$-^{32}\text{P}$]dCTP using the random primer method and was used to screen 10,000 plaques of the *L. major* expression library described above. Positive clones were compared by restriction enzyme digestion and the clone with the largest insert (pfl1-1) was chosen for subsequent analysis.

DNA sequence analyses were performed on an Applied Biosystems automated sequencer using Taq polymerase and dye coupled ddNTP terminators or dye-labeled sequencing primers. The complete sequence of the 2685 bp insert was determined using a combination of primer-directed sequencing and by sequencing a series of overlapping Exonuclease III deletion subclones generated using the Erase-a-base system (Promega, Madison, WI). The sequence of this insert is provided in SEQ ID NO:1, and the deduced amino acid sequence is provided in SEQ ID NO:2.

The complete insert of clone pfl1-1 was excised by digestion with *BamHI/KpnI* and was subcloned in frame into *BamHI/KpnI* digested pQE31 (QUIAGEN) to generate the construct pM151A. *E. coli* containing this construct inducibly expressed high levels of the *L. major* antigen encoded by pfl1-1 (designated as M15) with the addition of a 6-histidine tag at the amino terminus. Large volume cultures (500 ml) of *E. coli* host cells containing the pM151A construct were induced to express recombinant protein by the addition of 2mM IPTG at mid-log phase of growth. Growth was continued for 4 to 5 hours and bacteria were then pelleted and washed once with cold PBS. Bacteria were resuspended in 20 ml of lysis buffer (50 mM Na_2HPO_4 , pH 8.0, 300 mM NaCl, 10 mM β -mercaptoethanol) containing 20 mg of lysozyme and were lysed by a 1 hour incubation at 4°C followed by brief sonication. Insoluble material was removed by centrifugation at 10,000xg for 10 minutes and although the recombinant protein was found to be evenly distributed between the soluble and insoluble fractions the insoluble material was discarded at this point. Recombinant protein containing the amino terminal histidine tag was affinity purified using Ni-NTA resin (QUIAGEN) according to the manufacturer's recommendations. Briefly, 8 ml of

Ni-NTA resin resuspended in lysis buffer was added to the soluble lysate fraction and binding was conducted with constant mixing for 1 hour at 4°C. The mixture was then loaded into a gravity flow column and the non-binding material was allowed to flow through. The Ni-NTA matrix was washed 3 times with 25 ml of wash buffer (50 mM Na₂HPO₄, pH 6.0, 300 mM NaCl, 10 mM β-mercaptoethanol) and bound material was eluted in 25 ml of elution buffer (50 mM Na₂HPO₄, pH 5.0, 300 mM NaCl, 10mM β-mercaptoethanol). The eluted material was then dialyzed against 3 changes of PBS, sterile filtered and stored at -20°C. The purified recombinant protein was shown by SDS-PAGE analysis to be free of any significant amount of *E. coli* protein. A small number of bands of lower molecular weight were assumed to be proteolytic products of the *L. major* antigen based on their reactivity by western blot analysis. A high titre polyclonal antisera against M15 was generated in rabbits by repeated subcutaneous injection of recombinant protein. Western blot analysis of lysates from *L. major* promastigotes and amastigotes using this antisera indicated that the protein is constitutively expressed throughout the parasite lifecycle.

EXAMPLE 2

PREPARATION OF LDP23

This Example illustrates the preparation of a *Leishmania* antigen Ldp23, having the sequence provided in SEQ ID NO:4.

A. Purification of MHC Class II-associated Peptides from P388D1 Macrophages Infected with *L. donovani*

To ascertain that *in vitro* infection of macrophages would load their MHC class II molecules with parasite peptides, initial experiments were carried out to test the ability of *L. donovani*-infected macrophage cell line P388D1 to present parasite antigens to *L. donovani* specific T-cells. This macrophage cell line was chosen because it has the same H-2 haplotype as the BALB/c mouse, which is a strain of mouse

moderately susceptible to *L. donovani* infection and selected to conduct the *in vivo* experiments. Using a proportion of 3-5 parasites per cell and an initial incubation at room temperature for 4-6 hours follows by 37°C for 24-48 hours, close to 90% of the macrophages were infected. The level of MHC class II molecule expression, as
5 determined by FACS analysis, indicated that infection did not cause an effect on the levels of MHC class II expression when compared to non-infected control cells.

To test the ability of the *L. donovani*-infected P388D1 cells to present parasite antigens, macrophages were infected as indicated above and incubated at 26°C for 6 hours, and then as 37°C for either 24, 48 or 72 hours. At each of these time points
10 the non-adherent cells and free parasites were washed out and the adherent cells were mechanically dislodged, washed and fixed with paraformaldehyde. These cells were then used as antigen presenting cells (APCs) for purified lymph node T-cells from BALB/c mice immunized with *L. donovani* promastigotes. To generate these anti-*L. donovani* specific T-cells, BALB/c mice (H-2^d) of both sexes (The Jackson Laboratory,
15 Bar Harbor, ME) were immunized at 8 to 14 weeks of age in the rear foot pad with 5-10 x 10⁶ *L. donovani* promastigotes emulsified in complete Freund's adjuvant (CFA) (Difco Laboratories, Madison, MI) as described in Rodrigues et al., *Parasite Immunol.* 14:49 (1992). The draining lymph nodes were excised 8 days after the immunization and T-cells were purified in an anti-mouse Ig column to remove the B cells, as
20 described in Bunn-Moreno and Campos-Neto, *J. Immunol.* 127:427 (1981), followed by a passage through a Sephadex G10 column to remove the macrophages.

Stimulation index was calculated by dividing the cpm obtained for the cells cultured in the presence of infected P388D1 macrophages by the cpm obtained for the cells cultured in the presence of non-infected macrophages, but subjected to the
25 same conditions as the infected macrophages. The results shown Figure 1 indicate that *L. donovani*-infected P388D1 macrophage process parasite antigens and that optimal presentation occurs after 48 hours of infection. No stimulation of the T-cells by the non-infected macrophages was observed.

To isolate the MHC class II associated *L. donovani* peptides, P388D1
30 macrophages were infected with *L. donovani* promastigotes for an initial incubation of 6

hours at room temperature. The cultures were then transferred to 37°C for the remainder of the 48 hour incubation period. At a ratio of 3-5 parasites per macrophage nearly 90% of the macrophages were infected after 24 hours of incubation at 37°C.

The MHC class II molecules were then affinity-purified. Approximately
5 1.5×10^{10} *L. donovani*-infected or an equal number of non-infected P388D1
macrophages were used for each purification. The cells were harvested, washed with
PBS and incubated for 30 minutes in cold lysis buffer (PBS, 1% Nonidet P40, 25mM
iodoacetamide, 0.04% sodium azide, 1mM aprotinin and 1mM PMSF). The insoluble
material was removed by centrifugation at 40,000g for 1 hour and the supernatant was
10 recycled overnight at 4°C over a 5ml anti-MHC class II molecules (H-2^d) Sepharose
column (Protein G Sepharose column to which the monoclonal antibody MK-D6 has
been bound). Culture supernatants of MK-D6 hybridoma cells (American Type Culture
Collection, Rockville, MD) were employed as the source for anti-MHC class II (H-2^d)
monoclonal antibody. The column was washed with 50ml of lysis buffer and then with
15 50ml of PBS containing 0.5% octyl glucopyranoside detergent. Bound molecules were
eluted from the column with 1M acetic acid in 0.2% NaCl. The MHC/peptide
molecules were separated from the IgG (MK-D6 monoclonal antibody) using a
Centricon 100 filter unit (Amicon Division, W.R. Grace & Co., Beverly, MA). The
peptides were then dissociated from the class II molecules by the addition of acetic acid
20 to 2.5M, followed by separation using a Centricon 10 filter unit. The resulting peptide
preparation, present in the low molecular weight sample, was then dried using a speed
vac concentrator (Savant Instrument Inc., Farmingdale, NY).

The peptides were redissolved in 200µl of 0.05% TFA and separated by
reverse-phase high performance liquid chromatography (RP-HPLC) using a 2.1mm x
25 25cm Vydac C-18 column at a flow rate of 0.15ml/min employing a 1 to 30%
acetonitrile gradient (60 min) followed by a 30 to 60% gradient (30 min) and then a 60
to 80% gradient (90-110 min). Non-infected P388D1 cells were similarly processed to
serve as background control for endogenous MHC class II associated peptides. Figure 2
shows a representative experiment; four distinct peaks which are present only in the

material isolated from infected macrophages (panel B), and not in the material isolated from uninfected macrophages (panel A) are indicated.

Out of three independent peptide extractions, twenty five distinct HPLC peptide peaks were isolated from *L. donovani*-infected macrophages and were subjected to protein sequence analysis using automated Edman degradation on an Applied Biosystems 477 gas-phase protein sequencer. Protein sequence and amino acid analysis were performed by the W.M. Keck Foundation, Biotechnology Resource Laboratory, Yale University, New Haven, CT. In practically all determinations, no assignment could be made for the first position. Also, in most cases the definition of the amino acid residues of the 10-15 positions was based on the quantitative dominance of one residue over others. Using this approach, the sequences obtained for several peptides showed the presence of 3-6 different residues in many of the 10-15 sequence cycles analyzed for each determination, reflecting a mixture of peptides. In addition, sequences could not be obtained for some peaks because the peptides were blocked. Notwithstanding, three peptides sequences were determined. Amino-acid sequences were searched for identity with proteins in the GenBank database using the GENPETP, PIR and SWISSPROT programs. The sequence data base analysis revealed that one of the peptides was highly homologous to glyceraldehyde-3-phosphate dehydrogenase of various species. Another peptide had homology with elongation factor of several species, including *Leishmania*. The third sequence was not clearly related to any known proteins, and is shown below:

XQXPQ(L/K)VFDEXX (SEQ ID NO:11).

B. Cloning and Sequencing of the Ldp23 Gene

In order to retrieve the *L. donovani* protein that was processed into a peptide associated with the MHC class II molecules of infected macrophages, the peptide sequence of uncertain origin was chosen to guide the strategy for cloning the corresponding parasite gene. A DNA fragment was initially amplified from *L. donovani* promastigote cDNA by PCR. The sense primer was a peptide derived oligonucleotide (5' > GGAATTCCCCInCAGCTInGTInTTCGAC < 3') (SEQ ID NO:12) containing an *EcoRI* restriction endonuclease site (underlined). The bases were

selected following the preferential codon usage of *L. donovani*, as described in Langford et al., *Exp. Parasitol.* 74:360 (1992). Inosine was used for the residues of positions 4, 6 and 7 because of the low codon usage assurance for the corresponding amino acids. In addition, the carboxyl-terminal L-glutamic acid was not included for the design of the primer. The antisense primer was a poly-thymidine oligonucleotide (oligo dT, downstream primer) containing a *XhoI* restriction endonuclease site.

The gene fragment was amplified from a *L. donovani* promastigote cDNA preparation using the following reaction conditions: one cycle of 3 min at 94°C immediately followed by 35 cycles of 1 min at 94°C, 1 min at 45°C and 1 min at 72°C. The *L. donovani* cDNA was prepared from 5×10^7 washed promastigote forms harvested at the log growth phase (3 days culture). The cDNA was obtained using an Invitrogen cDNA cycle™ kit (Invitrogen Co., San Diego, CA). Oligonucleotide primers were synthesized by the DNA Synthesis Laboratory, Department of Pathology, Yale University School of Medicine.

The PCR products were analyzed by gel electrophoresis. Only one band of approximately 300 bp was obtained. This fragment was cloned and its sequence confirmed the sequence of the peptide-based primer including the glutamic acid codon, deliberately not included in the primer sequence.

The PCR amplified gene fragment was ligated into the pCR™ vector using the TA cloning system (Invitrogen Co., San Diego, CA). Transformants were selected in LB medium containing 100µg/ml ampicillin and the plasmid DNA was isolated using the Wizard™ Minipreps DNA purification kit (Promega Co., Madison, WI). Insert DNA was released with the restriction enzymes *EcoRI* and *XhoI* (New England Biolabs, Beverly, MA), purified from an agarose gel electrophoresis and labeled with ^{32}P using a random priming method (Megaprime Labeling Kit, Amersham Life Science, Buckinghamshire, England).

This DNA fragment was used as probe to screen a *L. donovani* promastigote cDNA library as described in Skeiky et al., *Infect. Immun.* 62:1643 (1994). An approximately 650 bp cDNA (Ldp23) was excised from the phagemid by *in vivo* excision using the Stratagene protocol. DNA sequencing was performed using the

Sequenase version 2 system (DNA sequencing kit) in the presence or absence of 7-deaza-GTP (United States Biochemical, Cleveland, OH). The sequence is provided as SEQ ID NO:3, and shows complete homology with the original 300 bp PCR fragment. A 525 bp open reading frame containing an ATG codon that follows the last 4 bases of the spliced leader sequence and 3 stop codons adjacent to the poly A tail was identified. This frame also codes the carboxyl terminal sequence (KVFDE) (SEQ ID NO:13) of the purified MHC class II associated peptide. The sequence analysis of the deduced protein sequence revealed one potential glycosylation site (Asn-Cys-Ser) at positions 68-70.

Sequence analysis was performed using the University of Wisconsin Genetics Computer Group Programs and the GenBank and EMBL data bases of protein and DNA sequences. The search for homology of the Ldp23 gene with known sequences revealed no significant homology.

C. Bacterial Expression and Purification of Recombinant Protein

The recombinant *L. donovani* peptide donor protein was produced in *E. coli* transformed with the pGEX 2T expression vector in which the Ldp23 gene was subcloned in frame. PCR was used to subclone the cloned gene in frame into the expression vector pGEX 2T. Primers containing the appropriate restriction site enzymes, initiation and termination codons were: 5' > GGATCCATGGTCAAGTCCCACTACATCTGC <3' (SEQ ID NO:14) for the upstream primer and 5' > GAATTCAGACCGGATAGAAATAAGCCAATGAAA <3' (SEQ ID NO:15) for the downstream primer (restriction sites of *Bam*HI and *Eco*RI are underlined respectively). PCR conditions were as indicated above for the amplification of the original peptide related DNA fragment. The template used was pBluescript plasmid containing the cloned gene from the cDNA library.

Overexpression of the recombinant fusion protein was accomplished by growing the transformed *E. coli* (DH5 α) and inducing the *tac* promoter with 1mM isopropyl- β -thiogalactopyranoside (IPTG) (Stratagene, La Jolla, CA). Cells were collected, centrifuged, and analyzed for the presence of the fusion protein by SDS-PAGE. A glutathione-S-transferase fusion protein of 43-44 kD was produced,

indicating a leishmanial protein of approximately 18 kD, as glutathione-S-transferase (GST) has a MW of 26 kD. However, the fusion protein was very insoluble and therefore could not be purified by affinity chromatography using a glutathione column. The use of low concentrations of detergents like SDS, sarcosyl, deoxycolate, and octyl-glucopyranoside during the extraction steps was efficient to solubilize the protein but unfortunately prevented its binding to the glutathione column. Other maneuvers, such as the growth of the *E. coli* and incubation and induction of the *tac* promoter with IPTG at 33°C, did not improve the protein solubility. However, the purification was achieved by preparative SDS-PAGE. The band was visualized with 0.1M KCl, cut and electroeluted from the gel followed by extensive dialysis against PBS and concentration on Centricon 10 filters.

Approximately 500µg of purified protein was obtained. The purified protein is shown in Figure 3. In panel A, *E. coli* (DH5α) transformed with the expression vector pGEX 2T containing the Ldp23 gene was grown in LB medium and the *tac* promoter was induced with IPTG for 3 hours. The cells were pelleted, resuspended in loading buffer and submitted to SDS-PAGE (10%) under reducing condition. The gel was stained with Coomassie blue. Lane 1 shows the uninduced *E. coli* and lane 2 shows the induced *E. coli*. The arrow indicates the recombinant protein. Panel B shows the protein prepared as in panel A and submitted to a preparative SDS-PAGE. The band corresponding to the overexpressed recombinant fusion protein was identified by KCl, cut out, electroeluted from the gel strip, dialyzed against PBS and submitted to analytical SDS-PAGE (12%). Numbers on the left side indicate the molecular weights of the markers. Attempts to further purify the leishmanial protein by cleaving it out from the fusion protein GST with thrombin were unsuccessful.

D. Expression of Ldp23

To ascertain that the Ldp23 peptide is expressed in *Leishmania* organisms, a Northern blot analysis was performed using RNA prepared from different promastigote growth phases (logarithmic and stationary) and from the amastigote form of these parasites.

The RNA was prepared from 2×10^7 parasite cells using the Micro RNA isolation kit (Stratagene, La Jolla, CA) according to the company's recommended instructions. RNA was prepared from *L. donovani* promastigotes (logarithmic growth phase); from *L. major* promastigotes (logarithmic and stationary growth phases); from 5 *L. amazonensis*, both promastigotes (logarithmic and stationary growth phases) and amastigotes purified from CBA/J infected mice; and from *L. pifanoi*, both promastigotes (logarithmic and stationary growth phases) and amastigotes (from axenic culture medium). *L. donovani* (1S strain), *L. amazonensis* (MHOM/BR/77/LTB0016), *L. major* (MHOM/IR/79/LRC-L251) and *L. pifanoi* (MHOM/VE/60/Ltrod) 10 promastigotes were grown and maintained at 26°C in Schneider's medium containing 20% FCS and 50µg/ml gentamicin. The amastigote forms of *L. amazonensis* were obtained by differential centrifugation of a "pus-like" foot pad lesion of a CBA/J mouse infected for 6 months with this parasite. *L. pifanoi* amastigotes were obtained from axenic culture as previously reported by Pan et al., *J. Euk. Microbiol.* 40:213 (1993).

15 The hybridization was carried out at 45°C in the presence of 50% formamide, 5x Denhardt's solution, 0.1% SDS, 100µg/ml single stranded salmon sperm DNA and 5x SSPE using 0.45µm Nytran membrane filters (Schleicher & Schuell, Keene, NH). The probe was the ^{32}P labeled Ldp23 gene.

Figure 4 shows that one single RNA band of 680 bp was observed for all 20 growth phases and forms of all tested *Leishmania*. Within Figure 4, the numbers 1, 2 and 3 refer to RNA obtained from promastigotes at the logarithmic growth phase, promastigotes at the stationary growth phase and amastigote forms, respectively, and the numbers on the left side indicate the molecular weights of the markers in base pairs. This result is consistent with the corresponding gene size (525 bp) and with the 25 molecular weight of the expressed protein and points to the ubiquitous distribution and expression of this gene within the genus *Leishmania*.

E. Induction of Anti-*L. donovani* Antibody Response in Mice and Rabbits by Purified Recombinant Protein

In order to evaluate the immunogenicity of the recombinant leishmanial protein, and to investigate its expression in the parasites, mice and rabbits were immunized with the GST-fusion protein in CFA. BALB/c mice were immunized in the rear foot pad with 5-10 μ g of protein emulsified in CFA. Protein concentration was
5 determined using the Bio-Rad Protein Assay reagent (Bio-Rad Laboratories, Richmond, CA). The mice were boosted 7 days later with 5-10 μ g of protein emulsified in incomplete Freund's adjuvant (IFA) inoculated into the peritoneal cavity. The mice were bled 7 days after the second immunization. New Zealand white rabbits (Millbrook Farm, Amherst, MA) were immunized according to the following protocol: one intra-
10 muscular (IM) injection of 25-30 μ g of purified recombinant protein emulsified in CFA into each thigh on day one; one IM injection of 25-30 μ g of purified protein emulsified in IFA into each shoulder on day 7; on day 15, 25-30 μ g of the purified protein in PBS was injected into the subcutaneous tissue. The rabbit was bled 7 days after the last immunization.

15 Sera were prepared and the anti-*Leishmania* antibody response was measured by Western blot analysis and by FACScan. In both cases *L. donovani* promastigotes were used as antigen. Approximately 2×10^6 *L. donovani* promastigotes were grown in Schneider's medium for 3 days (log phase), were washed with PBS, lysed with SDS-PAGE loading buffer and submitted to electrophoresis under reducing
20 conditions using a 15% polyacrylamide gel. The proteins were transferred onto 0.45 μ Immobilon-P transfer membrane (Millipore Co., Bedford, MA) using a wet-type electroblotter (Mini Trans-Blot Electrophoretic Transfer Cell, Bio Rad Life Science Division, Richmond, CA) for 2 hours at 50 V. The membranes were blocked overnight at room temperature with PBS containing 3% normal goat serum (NGS), 0.2% Tween-
25 20 and 0.05% sodium azide, followed by 3 washes with PBS. The blots were then incubated for 3-4 hours at 4°C with a 1/200 dilution of pre-immune rabbit serum (lane A, Figure 5) or with the same dilution of anti-fusion protein rabbit antiserum (lane B, Figure 5). The sera was previously absorbed 2x with non-viable desiccated *Mycobacterium tuberculosis* H-37 RA (Difco Laboratories, Detroit, MI) and were
30 diluted in PBS containing 1% NGS and 5% powdered non-fat bovine milk (Carnation,

Nestlé Food Company, Glendale, CA). The membranes were then washed with PBS, incubated for 1 hour at room temperature with goat anti-rabbit IgG antibody conjugated with alkaline phosphatase (Promega, Madison, WI); washed once with PBS and 2x with veronal buffer pH 9.4. The reaction was visualized using the substrate mixture 5-bromo-4-chloro-3-indoyl-phosphate and nitroblue tetrazolium (Kirkegaard & Perry Laboratories Inc., Gaithersburg, MD) according to the manufacturer's instructions.

Figure 5 shows that the rabbit anti-recombinant protein antiserum detects a single protein of 23 kDa (Ldp23) in the *Leishmania* crude extract antigen preparation. No bands were observed when an anti-GST antiserum was used (not shown). Moreover, the FACScan analysis (Figure 6) shows that the antibody induced by the recombinant Ldp23 reacts with intact live *L. donovani* promastigotes, thus pointing to a cell surface expression of this molecule on these organisms. The dotted line in Figure 6 shows the indirect immunofluorescence performed using pre-immune mouse serum and the solid line in Figure 6 shows the result obtained with mouse anti-GST-Ldp23 antiserum. Both sera were diluted at 1/100. Parasites were washed with staining buffer and incubated with FITC conjugated goat anti-mouse immunoglobulin antibody. Fluorescence intensity was analyzed by FACScan.

F. Recognition of Recombinant Ldp23 by *Leishmania*-Specific Lymph Node T-cells

To test the responsiveness of T-cells to the Ldp23 protein, two sets of experiments were performed. In the first experiment, lymph node T-cells (10^5 /well) from BALB/c mice immunized with *L. donovani* promastigotes (as described above) were stimulated to proliferate with 2×10^5 Mitomycin C-treated normal mononuclear spleen cells (APC) and pulsed with the purified recombinant fusion protein. Proliferation of T-cells was measured at 72 hours of culture. Values are expressed in Figure 7 as cpm and represent the mean of [3 H]TdR incorporation of triplicate cultures. Background cpm of cells (T cells + APC) cultured in the presence of medium alone was 1291. Figure 7 shows that *Leishmania* specific T-cells proliferate well and in a dose response manner to recombinant Ldp23. No response was observed when purified GST

was added instead of the recombinant fusion protein nor when lymph node T-cells from mice immunized with CFA alone were stimulated to proliferate in the presence of the Leishmanial fusion protein (not shown).

The recognition of the recombinant Ldp23 protein by *Leishmania*-specific T-cells was also tested using two murine models of leishmaniasis, the *L. major* highly susceptible BALB/c mice and the *L. amazonensis* susceptible CBA/J mice as described in Champs and McMahon-Pratt, *Infect. Immun.* 56:3272 (1988). These models were selected to investigate the cytokine pattern induced by Ldp23. In the mouse model of leishmaniasis, resistance is associated with Th 1 cytokines while susceptibility is linked to Th 2 responses.

Lymph node cells were obtained 3 weeks after the initiation of infection of BALB/c mice with *L. major* and the ability of these cells to recognize the recombinant Ldp23 was measured by proliferation and by the production of the cytokines IFN- γ and IL-4. 2×10^6 cells obtained from the draining popliteal lymph node of infected mice were cultured for 72 hours in the presence of recombinant Ldp23 or *Leishmania* lysate. The levels of IFN- γ and IL-4 in culture supernatants were measured by ELISA as previously described (Chatelain et al., *J. Immunol.* 148:1172 (1992), Curry et al., *J. Immunol. Meth.* 104:137 (1987), and Mossman and Fong, *J. Immunol. Meth.* 116:151 (1989)) using specific anti IFN- γ and IL-4 monoclonal antibodies (PharMingen, San Diego, CA).

Ldp23 did stimulate these cells to proliferate (not shown) and induced a typical Th 1 type of cytokine response as indicated by the production of high levels of IFN- γ (panel A of Figure 8) and no IL-4 (panel B of Figure 8). Stimulation of these cells with a *Leishmania* crude lysate yielded a mixed Th cytokine profile. Exactly the same pattern of cytokine production was obtained from the CBA/J mice infected with *L. amazonensis* (not shown). These results clearly indicate that Ldp23 is a powerful and selective activator of the Th 1 cytokines by mouse cells.

EXAMPLE 3

PREPARATION OF HSP83

This Example illustrates the preparation of a *Leishmania* antigen Hsp83,
5 having the sequence provided in SEQ ID NO:6.

A genomic expression library was constructed with sheared DNA from
L. braziliensis (MHOM/BR/75/M2903) in bacteriophage λ ZAP II (Stratagene, La Jolla,
CA). The expression library was screened with *Escherichia coli* preadsorbed serum
from an *L. braziliensis*-infected individual with ML. Immunoreactive plaques were
10 purified, and the pBSK(-) phagemid was excised by protocols suggested by the
manufacturer. Nested deletions were performed with exonuclease III to generate
overlapping deletions for single-stranded template preparations and sequencing. Single-
stranded templates were isolated following infection with VCSM13 helper phage as
recommended by the manufacturer (Stratagene, La Jolla, CA) and sequenced by the
15 dideoxy chain terminator method or by the *Taq* dye terminator system using the
Applied Biosystems automated sequencer model 373A.

Recombinant antigens produced by these clones were purified from 500
ml of isopropyl- β -D-thiogalactopyranoside (IPTG)-induced cultures as described in
Skeiky et al., *J. Exp. Med.* 176:201-211 (1992). These antigens were then assayed for
20 the ability to stimulate PBMC from *Leishmania*-infected individuals to proliferate and
secrete cytokine. Peripheral blood was obtained from individuals living in an area
(Corte de Pedra, Bahia, Brazil) where *L. braziliensis* is endemic and where
epidemiological, clinical, and immunological studies have been performed for over a
decade, and PBMC were isolated from whole blood by density centrifugation through
25 Ficoll (Winthrop Laboratories, New York, N.Y.). For *in vitro* proliferation assays, 2×10^5
to 4×10^5 cells per well were cultured in complete medium (RPMI 1640
supplemented with gentamicin, 2-mercaptoethanol, L-glutamine, and 10% screened
pooled A+ human serum; Trimar, Hollywood, Calif.) in 96-well flat-bottom plates with
or without 10 μ g of the indicated antigens per ml or 5 μ g of phytohemagglutinin per ml
30 (Sigma Immunochemicals, St. Louis, Mo.) for 5 days. The cells were then pulsed with

1 μ Ci of [3 H]thymidine for the final 18 h of culture. For determination of cytokine production 0.5 to 1 ml of PBMC was cultured at 1×10^6 to 2×10^6 cells per ml with or without the *Leishmania* antigens for 48 and 72 h.

The supernatants and cells were harvested and analyzed for secreted
5 cytokine or cytokine mRNAs. Aliquots of the supernatants were assayed for gamma interferon (IFN- γ), tumor necrosis factor alpha (TNF- α), interleukin-4 (IL-4), and IL-10 as described in Skeiky et al., *J. Exp. Med.* 181:1527-1537 (1995). For cytokine mRNA PCR analysis, total RNA was isolated from PBMC and cDNA was synthesized by using poly(dT) (Pharmacia, Piscataway, NJ) and avian myeloblastosis virus reverse
10 transcriptase. Following normalization to β -actin, diluted cDNA was amplified by PCR using *Taq* polymerase (Perkin-Elmer Cetus, Foster City, CA) with 0.2 μ M concentrations of the respective 5' and 3' external primers in a reaction volume of 50 μ l. The nucleotide sequences of the primary pairs and the PCR conditions used were as described in Skeiky et al., *J. Exp. Med.* 181:1527-1537 (1995). We verified that our
15 PCR conditions were within the semiquantitative range by initially performing serial dilutions of the cDNAs and varying the number of cycles used for PCR. Plasmids containing the human sequences for IL-2, IFN- γ , IL-4, IL-10, and β -actin were digested, and the DNA inserts were purified after separation on 1% agarose gels. Radiolabeled 32 P probes were prepared by the random priming method. PCR products were analyzed
20 by electrophoresis on 1.5% agarose gels, transferred to nylon membranes, and probed with the appropriate 32 P-labeled DNA insert.

A recombinant clone was identified in the above assays which, following sequence comparison of its predicted amino acid sequence with sequences of other proteins, was identified as a *Leishmania braziliensis* homolog of the eukaryotic 83 kD
25 heat shock protein (Lbhsp83). The sequence of the clone is provided in SEQ ID NO:5 and the deduced protein sequence is provided in SEQ ID NO:6. On the basis of the homology, this clone, designated Lbhsp83a, appears to lack the first 47 residues of the full length 703 amino acid residues. Lbhsp83 has an overall homology of 94% (91% identity and 3% conservative substitution), 91% (84% identity and 7% conservative
30 substitution) and 77% (61% identity and 16% conservative substitution) with *L.*

amazonensis hsp83, *T. cruzi* hsp83 and human hsp89, respectively. A second clone (designated Lbhsp83b), which contained the 43 kD C-terminal portion of hsp83 (residues 331 to 703) was also isolated. Figure 19 presents a comparison of the Lbhsp83 sequence with *L. amazonensis* hsp83(Lahsp83), *T. cruzi* hsp83 (Tchsp83) and human hsp89 (Huhsp89).

The results of proliferation assays using Lbhsp83a are shown in Table 1. Cells from all mucosal leishmaniasis (ML) patients proliferated strongly in response to Lbhsp83a, with stimulation indices (SIs) ranging from 19 to 558 (as compared to 20 to 1,634 for parasite lysate). Proliferation of PBMC from cutaneous leishmaniasis (CL) patients was variable and except for levels in two patients (IV and VII), levels were significantly lower than those of ML patients. By comparison, the proliferative responses of individuals with self-healing CL to Lbhsp83a were similar to those of individuals with ML. However, the responses of all six self-healing individuals to Lbhsp83 were consistently higher than those to Lbhsp83b. This suggests that PBMC from self-healing CL patients preferentially recognize one or more T-cell epitopes located within the amino portion of Lbhsp83.

Table 1

In vitro Proliferation of PMBC from *L. braziliensis*-infected Individuals
in Response to Lbhsp83

Group and Patient	Mean [³ H]thymidine incorporation [10 ³ cpm (SD)], SI with:		
	Lysate	Lbhsp83a	Lbhsp83b
ML			
I	41.3, (1.3), 294	32.5, (6.6), 221	46.7, (1.4), 318
II	44.2, (0.5), 104	20, (3.7), 47	36.7, (0.76), 86
III	27.4, (1.5), 150	8.1, (1.7), 44	9.9, (0.32), 54
IV	52.7, (3.3), 138	54.1, (6.2), 142	32.0, (1.3), 84
V	140.6, (7.6), 308	151.8, (57), 333	150.4, (7.9), 331
VI	15.8, (1.8), 20	21.3, (4.4), 28	14.4, (1.3), 19
VII	300.1, (9.4), 1634	102.1, (7.6), 558	41.7, (4.9), 228

Group and Patient	Mean [^3H]thymidine incorporation [10^3 cpm (SD)], SI with:		
	Lysate	Lbhsp83a	Lbhsp83b
CL			
I	0.26, (0.0), 1.5	0.57, (0.3), 3.3	0.43, (0.17), 3.3
II	55.63, (8.6), 218	0.42, (0.0), 1.6	0.8, (0.14), 3.2
III	0.39, (0.5), 4.0	3.4, (0.5), 9	2.6, (0.9), 6.6
IV	19.14, (1.3), 87	7.17, (0.6), 32	5.9, (0.9), 27
V	0.32, (0.2), 3.0	1.47, (0.5), 14	0.3, (0.1), 3.0
VI	0.77, (0.1), 4.7	1.44, (0.2), 9	1.3, (0.6), 8.0
VII	4.01, (1.0), 2.0	60.3, (8.5), 15	66.7, (3.9), 16.6
Self-healing CL			
I	19.7, (4.4), 94	61.3, (4.6), 293	5.0, (2.0), 24
II	0.6, (0.1), 6.5	7.0, (2.0), 79	1.2, (0.8), 13
III	59.6, (7.1), 519	49.4, (3.1), 429	21.4, (3.7), 186
IV	0.2, (0.1), 1.6	13.1, (1.7), 108	0.6, (0.1), 5
V	27.1, (2.0), 225	6.3, (2.6), 52	3.0, (1.5), 25
VI	130.3, (14), 340	28.2, (2.9), 74	7.7, (3.8), 20
Control (uninfected)			
I	0.19, (0.0), 1.4	0.18, (0.0), 1.3	0.40, (0.16), 2.8
II	0.31, (0.1), 1.7	0.19, (0.0), 1.0	0.27, (0.0), 1.5
III	0.44, (0.2), 4.1	0.48, (0.1), 5.0	0.51, (0.2), 5.2
IV	0.4, (0.1), 3.2	0.52, (0.2), 5.1	0.50, (0.1), 5.0

A more detailed analysis of cytokine patterns of PBMC from ML patients was performed by reverse transcriptase PCR. Cytokine mRNAs were evaluated in cells prior to culturing (Figure 9, lanes O) or following culturing in the absence (lanes -) or presence of the indicated antigen for 48 and 72 h. Figure 4A shows the results for five of the six ML patients whose PBMC were analyzed. In about half of the ML patients, noncultured (resting) PBMC had detectable levels of mRNA for IFN- γ , IL-2, and IL-4 but not IL-10. CL patient PBMC, however, had IL-10 mRNA in the resting state in addition to mRNAs for the other cytokines tested (Figure 4B). Following *in vitro* culture without antigen, the levels of mRNA for IFN- γ , IL-2, and IL-4 in resting cells from ML patients decreased to background levels while IL-10 mRNA levels increased. In contrast, PBMC of most CL patients had stable or increased IL-10

mRNA, while the mRNAs for IL-2, IFN- γ , and IL-4 were reduced to barely detectable levels in the absence of antigen stimulation.

In PBMC of three ML patients, stimulation with lysate resulted in increased expression of mRNA for IFN- γ , IL-2, and IL-4 but not IL-10. By comparison, both Lbhsp83 polypeptides elicited the production of mRNA for IFN- γ and IL-2 from all ML patient PBMC tested. In contrast, profiles of mRNA for IL-10 and IL-4 differed for the two hsp83 polypeptides. Lbhsp83a stimulated the production of IL-10 but not IL-4 mRNA (patients I, II, III, and IV), while Lbhsp83b stimulated the production of IL-4 but not IL-10 mRNA in all six patients.

All CL patients tested responded to both Lbhsp83 polypeptides as well as to the parasite lysate by upregulating the synthesis of mRNAs for IL-2 and IFN- γ , and in two of four patients (I and IV), the level of IL-4 mRNA also increased, indicating stimulation of both Th1 and Th2 cytokines. Interestingly and as in the case of ML patient uncultured PBMC which did not have detectable levels of IL-10 mRNA, Lbhsp83a and not Lbhsp83b stimulated PBMC from one CL patient (IV) to synthesize IL-10 mRNA. However, in the other three patients (I, II, and III) with resting levels of IL-10 mRNA, both rLbhsp83 polypeptides as well as the parasite lysate downregulated the expression of IL-10 mRNA.

PBMC supernatants were also assayed for the presence of secreted IFN- γ , TNF- α , IL-4, and IL-10. Cells from all ML and self-healing CL patients (seven and six patients, respectively) and from four of seven CL patients were analyzed for secreted IFN- γ following stimulation with both rLbhsp83 polypeptides, parasite lysate and Lbhsp70, an *L. braziliensis* protein homologous to the eukaryotic 70 kD heat shock protein (Figure 10A). In general, rLbhsp83a stimulated patient PBMC to secrete higher levels of IFN- γ than did rLbhsp83b (0.2 to 36 and 0.13 to 28 ng/ml, respectively). The presence of secreted IFN- γ correlated well with the corresponding mRNA detected by PCR

PBMC from four of five ML patients (I, II, V, and VII) had supernatant TNF- α levels (0.8 to 2.2 ng/ml) higher than those detected in cultures of PBMC from uninfected controls following stimulation with parasite lysate (Figure 10B). Similarly,

the same PBMC were stimulated by rLbhsp83 to produce levels of TNF- α in supernatant ranging from 0.61 to 2.9 ng/ml. Compared with those of uninfected controls, PBMC from three (I, V, and VI), five (I, II, IV, V, and VI), and two (II and V) of six individuals analyzed produced higher levels of TNF- α in response to parasite lysate, rLbhsp83a, and rLbhsp83b, respectively. The levels of TNF- α produced by PBMC from CL patients in response to parasite lysate were comparable to those produced by uninfected controls. However, rLbhsp83 stimulated TNF- α production in the PBMC of two of these patients. rLbhsp83a stimulated higher levels of TNF- α production than did rLbhsp83b. In the absence of antigen stimulation, only PBMC from ML patients (five of six) produced detectable levels of supernatant TNF- α (60 to 190 pg/ml).

In agreement with the IL-10 mRNA, IL-10 was detected by ELISA in the antigen-stimulated PMBC culture supernatants from ML and CL patients. The levels (49 to 190 pg) were significantly higher (up to 10-fold) following stimulation with rLbhsp83a compared with those after parallel stimulation of the same cells with rLbhsp83b (Figure 11). Parasite lysate also stimulated PMBC from some of the patients to produce IL-10. Although rLbhsp83 stimulated PMBC from uninfected individuals to produce IL-10, with one exception, the levels were lower than those observed with patient PMBC. IL-4 was not detected in any of the supernatants analyzed. Therefore, the level of any secreted IL-4 is below the detection limit of the ELISA employed (50 pg/ml). Taken together, the results demonstrate that a predominant Th1-type cytokine profile is associated with PMBC from *L. braziliensis*-infected individuals following stimulation with rLbhsp83 polypeptides.

To determine the correlation between the observed T-cell responses and antibody production to Lbhsp83, we compared the antibody (immunoglobulin G) reactivities to Lbhsp83 in sera from the three patient groups (Figure 12). The ELISA reactivities of ML patient sera with rLbhsp83a were comparable to those observed with parasite lysate, and in general, there was a direct correlation between ML patient anti-Lbhsp83 antibody titer and T-cell proliferation. Of 23 serum samples from ML patients analyzed, 22 were positive (~96%) with absorbance values of 0.20 to >3.0. Eleven of

the ML patient serum samples had optical density values that were >1 . In general, CL patients had significantly lower anti-Lbhsp83 antibody titers ($\bar{x} = 0.74$; standard error of the mean [SEM] = 0.1) compared to those of ML patients. Therefore, ML and CL patient anti-rhsp83 antibody titers correlated with their respective T-cell proliferative responses. Anti-rLbhsp83 antibody titers were significantly higher in patients with ML ($\bar{x} = 1.5$; SEM = 0.2) than in self-healing CL patients ($\bar{x} = 0.35$; SEM = 0.056), although their T-cell proliferative responses were similar. In fact, anti-Lbhsp83 antibody titers in serum from self-healing CL patients were comparable to those from uninfected controls ($\bar{x} = 0.24$; SEM = 0.028). By using 2 standard deviations greater than the mean absorbance value of uninfected control (0.484) as a criterion for positive reactivity to Lbhsp83, eight of nine of the self-healing patient serum samples tested were negative.

EXAMPLE 4

PREPARATION OF CLONES ENCODING LT-210

This Example illustrates the preparation of clones encoding portions of the *Leishmania* antigen Lt-210, and which has the sequence provided in SEQ ID NO:8.

An expression library was constructed from *L. tropica* (MHOM/SA/91/WR1063C) genomic DNA. The DNA was isolated by solubilizing *L. tropica* promastigotes in 10mM Tris-HCl, pH 8.3, 50mM EDTA, 1% SDS and treating with 100 μ g/ml RNaseA and 100 μ g/ml proteinase K. The sample was then sequentially extracted with an equal volume of phenol, phenol: chloroform (1:1), and Chloroform. DNA was precipitated by adding 0.1 volume of 3M sodium acetate (pH 5.2) and 2.5 volume 95% ethanol. The precipitate was resuspended in 10 μ M Tris, 1mM EDTA. DNA was sheared by passage through a 30-gauge needle to a size range of 2-6 kilobase, and was repaired by incubation with DNA polI in the presence of 100 μ M each dATP, dCTP, dGTP, and dTTP. *Eco*RI adapters were ligated to the DNA

fragments. After removal of unligated adapters by passage over a G-25 Sephadex™ column, the fragments were inserted in *EcoRI* cut Lambda ZapII (Stratagene, La Jolla, CA).

Approximately 43,000 pfu were plated and screened with sera isolated from viscerotropic leishmaniasis (VTL) patients. Sera from VTL patients were received from Drs. M. Grogl and A. Magill. The VTL patient group included eight individuals from whom parasites were isolated and cultured, seven of which had confirmed infection with *L. tropica*. Four other patients were culture negative, but were still considered to be infected based on either PCR analysis or a positive monoclonal antibody smear (Dr. Max Grogl, personal communication). Serum samples from the 11 infected patients were pooled and anti-*E. coli* reactivity removed by affinity chromatography (Sambrook et al., *supra*, p. 12.27-12.28). Lambda phage expressing reactive proteins were detected after antibody binding by protein A-horseradish peroxidase and ABTS substrate.

Three clones, Lt-1, Lt-2, and Lt-3, containing a portion of the Lt-210 gene were identified and purified. The clones ranged in size from 1.4 to 3.3 kb and encoded polypeptides of 75 kD, 70 kD, and 120 kD, respectively. These three clones contain partial sequences of the Lt-210 gene. Lt-1 and Lt-2 are overlapping clones and were chosen for further study.

The DNA sequences of Lt-1 and Lt-2 were determined. Exonuclease III digestion was used to create overlapping deletions of the clones (Heinikoff, *Gene* 28:351-359, 1984). Single strand template was prepared and the sequence determined with Applied Biosystems Automated Sequencer model 373A or by Sanger dideoxy sequencing. The sequence on both strands of the coding portion of Lt-1 clone was determined. The partial sequence of one strand of Lt-2 clone was determined.

SEQ ID NO:7 presents the DNA sequence of Lt-1, and SEQ ID NO:8 provides the predicted amino acid sequence of the open reading frame. The DNA sequence of the coding portion of the Lt-1 clone includes a repeated nucleotide sequence at the 5' portion of the clone containing eight copies of a 99 bp repeat, three copies of a 60 bp repeat unit, which is part of the larger 99 bp repeat, and 800 bp of

non-repeat sequence. The deduced amino acid sequence of the 99 bp repeat contains limited degeneracies. The mass of the predicted recombinant protein is 67,060 Daltons. A database search of PIR with the predicted amino acid sequence of the open reading frame yielded no significant homology to previously submitted sequences. Predicted
5 secondary structure of the repeat portion of the clone is entirely α -helical.

Sequence analysis of Lt-2 revealed that the 3' portion of the clone consisted of a mixture of 60 and 99 bp repeats that were identical, excepting occasional degeneracies, to the 60 and 99 bp repeats observed in Lt-1. Collectively, the sequencing data suggest that Lt-1 and Lt-2 are different portions of the same gene, Lt-2
10 being upstream of Lt-1, with possibly a small overlap.

Hybridization analysis confirmed that rLt-2 and rLt-1 contain overlapping sequences. Genomic DNAs of various *Leishmania* species were restricted with a variety of enzymes, separated by agarose gel electrophoresis, and blotted on Nytran membrane filter (Schleicher & Schuell, Keene, NH). Inserts from rLt-1 and rLt-
15 2 were labeled with ^{32}P -CTP by reverse transcriptase from random oligonucleotide primers and used as probes after separation from unincorporated nucleotides on a Sephadex G-50 column. Hybridizations using the rLt-1 or the rLt-2 probe are performed in 0.2M NaH_2PO_4 /3.6 M NaCl at 65°C, whereas hybridization using the rLt-lr probe is performed in 0.2 M NaH_2PO_4 /3.6 M NaCl/0.2 M EDTA at 60°C overnight.
20 Filters are washed in 0.075 M NaCl/0.0075 M sodium citrate pH 7.0 (0.15 M NaCl/0.0150 M sodium citrate for the Lt-lr probe), plus 0.5% SDS at the same temperature as hybridization.

Genomic DNA from a number of *Leishmania* species including *L. tropica* were analyzed by Southern blots as described above using the Lt-1, Lt-2, and
25 Lt-lr inserts separately as probes. Collectively, various digests of *L. tropica* DNA indicate that this gene has a low copy number. A similar, overlapping pattern was observed using either the Lt-1 or Lt-2 insert as a probe, consistent with the premise that these two clones contain sequences near or overlapping one another. In addition, sequences hybridizing with these clones are present in other *Leishmania* species.

L. tropica isolates have limited heterogeneity. Southern analyses of digested genomic DNA from four *L. tropica* parasite strains isolated from VTL patients and three *L. tropica* parasite strains isolated from CL cases (two human, one canine) were performed. The Lt-lr insert described below was labeled and used as a probe. The
5 seven different *L. tropica* isolates yielded similar intensities and restriction patterns, with only a single restriction fragment length polymorphism among the isolates. These data, along with Southern analyses with additional enzymes, indicate limited heterogeneity in this region among the *L. tropica* isolates.

The recombinant proteins of Lt-1 and Lt-2 were expressed and purified.
10 The nested deletion set of Lt-1 formed for sequencing included a clone referred to as Lt-lr, which contains one and one-third repeats. This polypeptide was also expressed and purified. *In vivo* excision of the pBluescript SK⁻ phagemid from Lambda Zap II was performed according to the manufacturer's protocol. Phagemid virus particles were used to infect *E. coli* XL-1 Blue. Production of protein was induced by the addition of
15 IPTG. Protein was recovered by first lysing pellets of induced bacteria in buffer (LB, 50 mM Tris-HCl, pH 8.0, 100 mM NaCl, 10 mM EDTA) using a combination of lysozyme (750µg/mL) and sonication. rLt-1, rLt-2, and rLt-lr, were recovered from the inclusion bodies after solubilization in 8M urea (rLt-1 and rLt-2) or 4M urea (rLt-lr). Proteins rLt-1 and rLt-2 were enriched and separated by precipitation with 25%-40%
20 ammonium sulfate and rLt-lr was enriched by precipitation with 10%-25% ammonium sulfate. The proteins were further purified by preparative gel electrophoresis in 10% SDS-PAGE. Recombinant proteins were eluted from the gels and dialyzed in phosphate-buffered saline (PBS). Concentration was measured by the Pierce (Rockford, IL) BCA assay, and purity assessed by Coomassie blue staining after SDS-
25 PAGE.

EXAMPLE 5

PREPARATION OF LbeIF4A

This example illustrates the molecular cloning of a DNA sequence
5 encoding the *L. braziliensis* ribosomal antigen LbeIF4A.

A genomic expression library was constructed with sheared DNA from
L. braziliensis (MHOM/BR/75/M2903) in bacteriophage λ ZAPII (Stratagene, La Jolla,
CA). The expression library was screened with *E. coli*-preadsorbed patient sera from an
L. braziliensis-infected individual with mucosal leishmaniasis. Plaques containing
10 immunoreactive recombinant antigens were purified, and the pBSK(-) phagemid
excised using the manufacturer's protocols. Nested deletions were performed with
Exonuclease III to generate overlapping deletions for single stranded template
preparations and sequencing. Single stranded templates were isolated following
infection with VCSM13 helper phage as recommended by the manufacturer (Stratagene,
15 La Jolla, CA) and sequenced by the dideoxy chain terminator method or by the Taq dye
terminator system using the Applied Biosystems Automated Sequencer Model 373A.

The immunoreactive recombinant antigens were then analyzed in patient
T-cell assays for their ability to stimulate a proliferative and cytokine production, as
described in Examples 7 and 8 below.

20 A recombinant clone was identified in the above assays which, following
sequence comparison of its predicted amino acid sequence with sequences of other
proteins, was identified as a *Leishmania braziliensis* homolog of the eukaryotic
initiation factor 4A (eIF4A). The isolated clone (pLeIF.1) lacked the first 48 amino
acid residues (144 nucleotides) of the full length protein sequence. The pLeIF.1 insert
25 was subsequently used to isolate the full length genomic sequence.

SEQ ID NO:9 shows the entire nucleotide sequence of the full-length
LbeIF4A polypeptide. The open reading frame (nucleotides 115 to 1323) encodes a 403
amino acid protein with a predicted molecular weight of 45.3 kD. A comparison of the
predicted protein sequence of LbeIF4A with the homologous proteins from tobacco
30 (TeIF4A), mouse (MeIF4A), and yeast (YeIF4A) shows extensive sequence homology,

with the first 20-30 amino acids being the most variable. The lengths (403, 413, 407, and 395 amino acids), molecular weights (45.3, 46.8, 46.4, and 44.7 kDa), and isoelectric points (5.9, 5.4, 5.5, and 4.9) of LbeIF4A, TeIF4A, MeIF4A and YeIF4A, respectively, are similar. LbeIF4A shows an overall homology of 75.5% (57% identity, 18.5% conservative substitution) with TeIF4A, 68.6% (50% identity, 18.6% conservative substitution) with MeIF4A and 67.2% (47.6% identity, 19.6% conservative substitution) with YeIF4A.

EXAMPLE 6

PREPARATION OF SOLUBLE *LEISHMANIA* ANTIGENS

This Example illustrates the preparation of soluble *Leishmania* antigens from an *L. major* culture supernatant. *L. major* promastigotes were grown to late log phase in complex medium with serum until they reached a density of $2-3 \times 10^7$ viable organisms per mL of medium. The organisms were thoroughly washed to remove medium components and resuspended at $2-3 \times 10^7$ viable organisms per mL of defined serum-free medium consisting of equal parts RPMI 1640 and medium 199, both from Gibco BRL, Gaithersburg, MD. After 8-12 hours, the supernatant was removed, concentrated 10 fold and dialyzed against phosphate-buffered saline for 24 hours. Protein concentration was then determined and the presence of at least eight different antigens confirmed by SDS-PAGE. This mixture is referred to herein as "soluble *Leishmania* antigens."

EXAMPLE 7COMPARISON OF INTERLEUKIN-4 AND INTERFERON- γ PRODUCTION
STIMULATED BY *LEISHMANIA* ANTIGENS

5 This Example illustrates the immunogenic properties of the antigens prepared according to Examples 1, 2, 5 and 6, as determined by their ability to stimulate IL-4 and IFN- γ in lymph node cultures from infected mice and in human PBMC preparations. Lymph node cultures for use in these studies were prepared from *L. major*-infected BALB/c mice 10 days after infection, as described in Example 2.

10 PBMC were prepared using peripheral blood obtained from individuals with cured *L. donovani* infections who were immunologically responsive to *Leishmania*. Diagnosis of the patients was made by clinical findings associated with at least one of the following: isolation of parasite from lesions, a positive skin test with *Leishmania* lysate or a positive serological test. Uninfected individuals were identified based on a lack of

15 clinical signs or symptoms, a lack of history of exposure or travel to endemic areas, and the absence of a serological or cellular response to *Leishmania* antigens. Peripheral blood was collected and PBMC isolated by density centrifugation through Ficoll™ (Winthrop Laboratories, New York).

Culture supernatants were assayed for the levels of secreted IL-4 and

20 IFN- γ . IFN- γ was quantitated by a double sandwich ELISA using mouse anti-human IFN- γ mAb (Chemicon, Temucula, CA) and polyclonal rabbit anti-human IFN- γ serum. Human rIFN- γ (Genentech Inc., San Francisco, CA) was used to generate a standard curve. IL-4 was quantitated in supernatants by a double sandwich ELISA using a mouse anti-human IL-4 mAb (M1) and a polyclonal rabbit anti-human IL-4 sera (P3).

25 Human IL-4 (Immunex Corp., Seattle, WA) was used to generate a standard curve ranging from 50 pg/ml to 1 ng/ml.

Figures 13A and 13B, illustrate the mean level of secreted IL-4 and IFN- γ , respectively, 72 hours after addition of 10 μ g/mL of each of the following antigens to a lymph node culture prepared as described above: soluble *Leishmania* antigen (*i.e.*, an

30 extract prepared from ruptured promastigotes which contains membrane and internal

antigens (SLA)), Ldp23, LbeIF4A (LeIF), Lbhsp83, M15 and LmeIF (the *L. major* homolog of LbeIF4A). The levels of secreted IL-4 and IFN- γ in medium alone (*i.e.*, unstimulated) are also shown. While SLA elicits a predominantly Th2 response from lymph node cells of Leishmania-infected mice, Ldp23, LbeIF4A, Lbhsp83 and M15
5 elicited relatively little IL-4 and large amounts of IFN- γ , consistent with a Th1 response profile.

Figure 14 shows the level of secreted IFN- γ in culture filtrate from infected and uninfected human PBMC preparations 72 hours after addition of 10 μ g/mL *L. major* lysate, M15 or L-Rack, an immunodominant leishmanial antigen in murine
10 leishmaniasis. Similarly, Figure 15 illustrates the level of secreted IFN- γ in culture filtrate from infected and uninfected human PBMC preparations 72 hours after addition of 10 μ g/mL *L. major* lysate, soluble *Leishmania* antigens (prepared as described in Example 6) or L-Rack. These results indicate that M15 and soluble *Leishmania* antigens, but not L-Rack, are potent stimulators of IFN- γ production in patient PBMC,
15 but not in PBMC obtained from uninfected individuals. Thus, M15 and soluble *Leishmania* antigens elicit a dominant Th1 cytokine profile in both mice and humans infected with *Leishmania*.

20

EXAMPLE 8

COMPARISON OF PROLIFERATION STIMULATED BY *LEISHMANIA* ANTIGENS

This Example illustrates the immunogenic properties of the antigens prepared according to Examples 1, 2, 5 and 6, as determined by their ability to stimulate
25 proliferation in lymph node cultures from infected mice and in human PBMC preparations.

For *in vitro* proliferation assays, 2 - 4 x 10⁵ cells/well were cultured in complete medium (RPMI 1640 supplemented with gentamycin, 2-ME, L-glutamine, and 10% screened pooled A+ human serum; Trimar, Hollywood, CA) in 96-well flat
30 bottom plates with or without 10 μ g/ml of the indicated antigens or 5 μ g/ml PHA

(Sigma Immunochemicals, St. Louis, MO) for five days. The cells were then pulsed with 1 μ Ci of [3 H] thymidine for the final 18 hours of culture.

Figure 16 illustrates the proliferation observed after addition of 10 μ g/mL or 20 μ g/mL of each of the following antigens to a lymph node culture prepared as described in Example 7: SLA, Ldp23, LbelF4A, Lbhsp83, and M15. The level of proliferation without the addition of antigen is also shown. Data are represented as mean cpm. These results demonstrate that a variety of leishmanial antigens are capable of stimulatory lymph node cell proliferation from *Leishmania*-infected mice.

Figures 17 and 18 illustrate the proliferation observed in human PBMC preparations from *Leishmania*-immune and uninfected individuals following the addition of 10 μ g/mL M15 and soluble *Leishmania* antigens, respectively. These values are compared to the proliferation observed following the addition of culture medium, *L. major* lysate or L-Rack. The results show that M15 and soluble *Leishmania* antigens stimulate proliferation in *Leishmania*-immune PBMC, but not in PBMC obtained from uninfected individuals, demonstrating that M15 and soluble antigens (but not L-Rack) are recognized by PBMC from individuals immune to *Leishmania* due to a previous infection.

EXAMPLE 9

PREPARATION OF LMSP1A AND LMSP9A

This Example illustrates the preparation of two soluble *Leishmania* antigens, Lmsp1a and Lmsp9a.

A. Purification of Lmsp1a and Lmsp9a from a mixture of soluble *L. major* antigens

A high titer rabbit sera was raised against *L. major* soluble antigens, prepared as described above in Example 6. Specifically, a New Zealand white rabbit was immunized subcutaneously at multiple sites with 180 μ g of *L. major* soluble antigens in a suspension containing 100 μ g muramyl dipeptide and 50 % incomplete

Freund's adjuvant. Six weeks later the rabbit was given a subcutaneous boost of 100 µg of the same soluble antigen preparation in incomplete Freund's adjuvant. This was followed by two intravenous boosts spaced two weeks apart, each with 100 µg of the soluble antigen preparation. Sera was collected from the rabbit 11 days after the final
5 boost.

Anti *E. coli* antibody reactivities were removed from the rabbit sera by pre-adsorbing on nitrocellulose filters containing lysed *E. coli*. Adsorbed sera were evaluated by Western blot analysis using 10 µg *Leishmania* promastigote lysate (lane 1) and 1 µg soluble *L. major* antigen mixture (lane 2). As shown in Figure 20, the rabbit
10 sera was found to be reactive with seven dominant antigens of the soluble *L. major* antigen mixture with molecular weights ranging from 18 to >200 kDa. A four times longer exposure of the same blot revealed three additional immunoreactive species with molecular weights less than 18 kDa. The same sera reacted with approximately 10 antigens of the promastigote lysate, but with a pattern significantly different from that
15 observed with the soluble *L. major* antigens (Figure 20). This is suggestive of potential post-translational modification of the same antigen before (intracellular localization) and after secretion/shedding. Such modifications may include cleavage of a leader sequence and/or the addition of carbohydrate molecules to the secreted/shed antigens.

The rabbit sera described above was subsequently used to screen an *L. major* cDNA expression library prepared from *L. major* promastigote RNA using the
20 unidirectional Lambda ZAP (uni-ZAP) kit (Stratagene) according to the manufacturer's protocol. A total of 70,000 pfu of the amplified cDNA library was screened with the rabbit sera at a 1:250 dilution. Nineteen positive clones were confirmed in the tertiary screening. The phagemid were excised and DNA from each of the 19 clones was
25 sequenced using a Perkin Elmer/Applied Biosystems Division automated sequencer Model 373A. All 19 clones were found to represent two distinct sequences, referred to as Lmsp1a and Lmsp9a. The determined cDNA sequences for Lmsp1a and Lmsp9a are provided in SEQ ID NO: 19 and 21, respectively, with the corresponding amino acid sequences being provided in SEQ ID NO: 20 and 22, respectively.

B. Characterization of Lmsp1a and Lmsp9a

Fig. 21 shows the full-length cDNA (SEQ ID NO: 19) and predicted amino acid sequence (SEQ ID NO: 20) for the antigen Lmsp1a. The EcoRI/XhoI insert is 1019 bp long and contains the following features: a) the last 17 nt of the spliced leader sequence characteristic of all trypanosoma nuclearly encoded mRNA; b) 39 nt of 5' untranslated sequence; c) an open reading frame of 453 nt long coding for a 151 deduced amino acid sequence with a predicted molecular mass of 16.641 kDa; and d) 471 nt of 3' untranslated sequence terminating with a poly A tail. The predicted amino acid sequence contains three potential phosphorylation sites at amino acid residues 3, 85 and 102. In addition, Lmsp1a contains an RGD sequence at residue 104, a sequence that may play a role in parasite invasion of the macrophage. RGD sequences have been shown to mediate the binding of various adhesion proteins to their cell surface receptors. There is no obvious leader sequence (secretory signal) at the amino terminal portion suggesting that the protein might be shed or excreted. Lmsp1a appears to be one of the most abundant antigens found in the culture supernatant of live promastigote, since 17 of the 19 clones contain sequences of variable lengths identical to Lmsp1a.

Comparison of the amino acid sequence of Lmsp1a with known sequences using the DNA STAR system (Version 87) revealed that Lmsp1a shares between 65% to 70% homology with the eukaryotic nucleoside diphosphate kinase protein, also referred to in the mouse and human as a tumor metastasis inhibitor gene.

Southern blot analysis of genomic DNA from *L. major* (Friedlander strain) digested with a panel of restriction enzymes (lanes 1 to 7) and six other *Leishmania* species of different geographic locations digested with PstI (lanes 8 to 13) using the full-length cDNA insert of Lmsp1a, demonstrated that Lmsp1a is present in all the species characterized with a high degree of conservation (Fig. 22). This suggests evolutionary significance for the maintenance of Lmsp1a and the existence of homologous species among all the *Leishmania* species.

The remaining two cDNA clones isolated from the soluble *L. major* antigen mixture represent identical sequences (referred to as Lmsp9a; SEQ ID NO: 21), suggesting that the two copies resulted from amplification of the primary library.

Sequencing of the Lmsp9a cDNA revealed that the clone does not contain the full length 5' sequence since it is lacking both the spliced leader and 5' untranslated sequences. The 3' end of the cDNA contains a poly A stretch, as would be expected for a *Leishmania* mRNA. Of the predicted translated sequence (SEQ ID NO: 22), 34 of the
5 201 amino acids (17%) represent cysteine residues. Comparison of the predicted protein sequence with those of known proteins as described above, revealed some homology with other cysteine rich proteins such as the major surface trophozoite antigen of *Giardia lamblia* and furin proteases.

10

EXAMPLE 10

PREPARATION AND CHARACTERIZATION OF MAPS-1A

This Example illustrates the preparation and characterization of the
15 *Leishmania* antigen MAPS-1A (SEQ ID NO: 24).

A pool of sera was obtained from 5 BALB/c mice that had been given a primary immunization and two boosts with crude *L. major* promastigote culture supernatant as described below in Example 12. These mice were subsequently shown to be protected when challenged with a dose of live *L. major* promastigotes generally
20 found to be lethal. The mouse sera thus obtained were used to screen an *L. major* amastigote cDNA expression library prepared as described in Example 1. Several seroreactive clones were isolated and sequenced using a Perkin Elmer/Applied Biosystems Division automated sequencer Model 373A (Foster City, CA).

One of these clones, referred to herein as MAPS-1A, was found to be
25 full-length. Comparison of the cDNA and deduced amino acid sequences for MAPS-1A (SEQ ID Nos: 23 and 24, respectively) with known sequences in the gene bank using the DNA STAR system revealed no significant homologies to known *Leishmania* sequences, although some sequence similarity was found to a group of proteins, known as thiol-specific antioxidants, found in other organisms.

Recombinant MAPS-1A protein having an amino-terminal HIS-Tag was prepared using a high level *E. coli* expression system and recombinant protein was purified by affinity chromatography as described in Example 1. Southern blot analysis of genomic DNA from *L. major* digested with a panel of restriction enzymes, seven
5 other *Leishmania* species digested with PstI, and two other infectious-disease pathogens (*T. cruzi* and *T. brucei*), using the full length insert of MAPS-1A, demonstrated that MAPS-1A is present in all eight *Leishmania* species tested (Figure 23). Northern blot analysis of *L. major* promastigote and amastigote RNAs indicated that MAPS-1A is constitutively expressed.

10 Using oligonucleotide primers (SEQ ID NOs:27 and 28) based on the MAPS-1A cDNA sequence provided in SEQ ID NO: 23, the corresponding gene was isolated from *L. tropica* by means of PCR (using 30 cycles of the following temperature step sequence: 94 °C, 1 minute; 50 °C, 1 minute; 72 °C, 1 minute) The determined cDNA sequence for the *L. tropica* MAPS-1A protein is provided in SEQ ID
15 NO: 25, with the corresponding amino acid sequence being provided in SEQ ID NO: 26.

The ability of recombinant MAPS-1A to stimulate cell proliferation was investigated as follows. PBMC from 3 *L. braziliensis*-infected patients having active mucosal leishmaniasis, from 4 patients post kala-azar infection (previously infected
20 with *L. chagasi* and/or *L. donovani*) and from 3 uninfected-individuals were prepared as described above in Example 7. The ability of MAPS-1A to stimulate proliferation of these PBMC was determined as described in Example 8 above. As shown in Figure 24, significant levels of MAPS-1A specific PBMC proliferation were seen in 2 of the 7 *Leishmania* patients.

25 The ability of MAPS-1A to stimulate proliferation in mice lymph node cultures was determined as described in Example 8. Figure 25 shows the amount of proliferation stimulated by MAPS-1A (at 25 µg/ml, 5 µg/ml and 1 µg/ml) as compared to that stimulated by the positive control ConA and by crude *L. major* promastigote supernatant proteins, 20 days post-infection with *L. major*. Cells isolated 20 days post-

infection were highly responsive to MAPS-1A, whereas cells isolated 10 days post-infection were unresponsive.

EXAMPLE 11

5 IMMUNOREACTIVITY OF SOLUBLE *LEISHMANIA* ANTIGENS WITH SERA FROM *LEISHMANIA*-INFECTED PATIENTS

The reactivity of MAPS-1A with sera from uninfected individuals, from human leishmaniasis patients with cutaneous infection, from human patients with acute
10 visceral leishmaniasis, and from *L. major*-infected BALB/c mice was determined as follows.

Assays were performed in 96-well plates coated with 200 ng antigen diluted to 50 μ L in carbonate coating buffer, pH 9.6. The wells were coated overnight at 4 °C (or 2 hours at 37 °C). The plate contents were then removed and the wells were
15 blocked for 2 hours with 200 μ L of PBS/1% BSA. After the blocking step, the wells were washed five times with PBS/0.1% Tween 20TM. 50 μ L sera, diluted 1:100 in PBS/0.1% Tween 20TM/0.1% BSA, was then added to each well and incubated for 30 minutes at room temperature. The plates were then washed again five times with PBS/0.1% Tween 20TM.

20 The enzyme conjugate (horseradish peroxidase - Protein A, Zymed, San Francisco, CA) was then diluted 1:10,000 in PBS/0.1% Tween 20TM/0.1% BSA, and 50 μ L of the diluted conjugate was added to each well and incubated for 30 minutes at room temperature. Following incubation, the wells were washed five times with PBS/0.1% Tween 20TM. 100 μ L of tetramethylbenzidine peroxidase (TMB) substrate
25 (Kirkegaard and Perry Laboratories, Gaithersburg, MD) was added, undiluted, and incubated for about 15 minutes. The reaction was stopped with the addition of 100 μ L of 1 N H₂SO₄ to each well, and the plates were read at 450 nm.

As shown in Figure 26, approximately 50% of the samples from human leishmaniasis patients showed reactivities with recombinant MAPS-1A substantially
30 above background. Figure 27 shows the reactivity of MAPS-1A with increasing

dilutions of sera from BALB/c mice previously administered either (i) saline solution; (ii) the adjuvant *B. pertussis*; (iii) soluble *Leishmania* antigens plus *B. pertussis*; (iv) live *L. major* promastigotes; or (v) soluble *Leishmania* antigens plus *B. pertussis* followed by live *L. major* promastigotes (as described below in Example 12).

- 5 Considerably higher absorbances were seen with sera from mice infected with live *L. major* promastigotes and with mice infected with live *L. major* promastigotes following immunization with soluble *Leishmania* antigens plus *B. pertussis*, than with sera from the other three groups of mice, indicating that anti-MAPS-1A antibody titers increase following *Leishmania* infection.

10

EXAMPLE 12

USE OF *LEISHMANIA* ANTIGENS FOR VACCINATION AGAINST *LEISHMANIA* INFECTION

- 15 This example illustrates the effectiveness of *Leishmania* antigens in conferring protection against disease in the experimental murine leishmaniasis model system. For a discussion of the murine leishmaniasis model system see, for example, Reiner *et al. Annu. Rev. Immunol.*, 13:151-77, 1995.

- The effectiveness of (i) crude soluble *Leishmania* antigens, (ii) MAPS-
20 1A, and (iii) a mixture of Ldp23, LbeIF4A and M15, as vaccines against *Leishmania* infection was determined as follows. BALB/c mice (5 per group) were immunized intra-peritoneally three times at biweekly intervals with either (i) 30 µg crude soluble *Leishmania* antigens, (ii) 20 µg MAPS-1A or (iii) a mixture containing 10 µg each of LeIF, Ldp23 and M15, together with 100 µg of the adjuvant *C. parvum*. Two control
25 groups were immunized with either saline or *C. parvum* alone. Two weeks after the last immunization, the mice were challenged with 2×10^5 late-log phase promastigotes of *L. major*. Infection was monitored weekly by measurement of footpad swelling. The amount of footpad swelling seen in mice immunized with either crude soluble *Leishmania* antigens, a mixture of Ldp23, LbeIF4A and M15 (Figure 28), or MAPS-1A
30 (Figure 29) was significantly less than that seen in mice immunized with *C. parvum*

alone. These results demonstrate that the *Leishmania* antigens of the present invention are effective in conferring protection against *Leishmania* infection.

EXAMPLE 13

5 ISOLATION OF DNA ENCODING FOR SOLUBLE ANTIGENS FROM AN *L. MAJOR* GENOMIC DNA LIBRARY

This example illustrates the isolation of seven soluble *Leishmania* antigen genes from an *L. major* genomic DNA library.

10 An *L. major* genomic DNA expression library was prepared from *L. major* promastigotes using the unidirectional Lambda ZAP (uni-ZAP) kit (Stratagene) according to the manufacturer's protocol. This library was screened with a high titer rabbit sera raised against *L. major* soluble antigens, as described above in Example 9. Seven positive clones were identified. The phagemid were excised and DNA from
15 each of the seven clones was sequenced using a Perkin Elmer/Applied Biosystems Division automated sequencer Model 373A. The DNA sequences for these antigens, referred to as LmgSP1, LmgSP3, LmgSP5, LmgSP8, LmgSP9, LmgSP13, LmgSP19, are provided in SEQ ID NO:29-35, respectively, with the corresponding amino acid sequences being provided in SEQ ID NO: 36-42, respectively. LmgSP13 was found to
20 contain a 39 amino acid repeat sequence shown in SEQ ID NO:43.

Subsequent studies resulted in the isolation of a full-length sequence for LmgSP9. The full-length DNA sequence is provided in SEQ ID NO: 54, with the corresponding predicted amino acid sequence being provided in SEQ ID NO: 55. The amino acid sequence was found to contain six 14 amino acid repeat units (SEQ ID NO:
25 56), with each unit being further divided into two 7 amino acid units, provided in SEQ ID NO: 57 and 58.

Comparison of the DNA and amino acid sequences for the isolated antigens as described above, revealed no significant homologies to LmgSP1, LmgSP3, and LmgSP13. LmgSP5 was found to be related to the known PSA2 family. LmgSP8
30 was found to bear some homology to a sequence previously identified in *E. coli* (2-

succinyl-6-hydroxy-2,4-cyclohexadiene-1-carboxylic acid synthase). LmgSP9 and LmgSP19 were found to be homologous to a *L. major* hydrophilic surface protein referred to as Gene B (Flinn, H.M. et al. *Mol. Biochem. Parasit.* 65:259-270, 1994), and to ubiquitin, respectively. To the best of the inventors' knowledge, none of these
5 antigens have been previously shown to elicit T or B cell responses.

The reactivity of recombinant LmgSP9 with sera from patients with visceral leishmaniasis, (from both Sudan and Brazil) and from normal donors was evaluated by ELISA as described above. The absorbance values were compared with those obtained using the known *Leishmania* antigen K39 described above, with *L.*
10 *chagasi* lysate being employed as a positive control. Representative results of these assays are provided below in Table 2, wherein all the patients from Brazil and those from the Sudan designated as "VL" were inflicted with visceral leishmaniasis. The results demonstrated that LmgSP9 specifically detects antibody in most individuals with visceral leishmaniasis, regardless of geographical location. In several cases, the
15 absorbance values of the antibody reactivity to LmgSP9 were comparable to that observed with K39. In addition, LmgSP9 detected several cases of leishmaniasis that were not detected using K39. These results indicate that LmgSP9 can be used to complement the reactivity of K39.

Table 2

REACTIVITY OF LMGSP9 WITH SERA FROM LEISHMANIA PATIENTS

Patient No.	<i>L. chagasi</i> lysate	K39	LmgSP9
Sudanese samples:			
B19	1.067	0.306	0.554
B25	1.884	3.435	0.974
B43	1.19	3.225	0.86
B47	2.405	2.892	0.375
B50	0.834	0.748	0.432
B58	0.921	0.235	0.92
B63	1.291	0.303	0.764
B70	0.317	0.089	3.056
VL4	1.384	3.035	2.965
VL11	0.382	0.144	0.142
VL12	0.277	0.068	0.098
VL13	0.284	0.12	0.194
Brazilian samples:			
105	3.508	3.53	0.374
106	2.979	3.373	2.292
107	2.535	3.444	0.46
109	1.661	3.415	3.319
111	3.595	3.537	0.781
112	2.052	3.469	0.63
113	3.352	3.429	0.963
114	2.316	3.437	1.058
115	2.073	3.502	1.186
116	3.331	3.461	0.96
Normal Donors:			
129	0.157	0.104	0.08
130	0.195	0.076	0.095
131	0.254	0.134	0.086
132	0.102	0.035	0.043

In order to obtain a higher specificity for the detection of antibodies in sera from visceral leishmaniasis patients, a homologue of LmgSP9 was isolated from *L. chagasi*, one of the causative agents of visceral leishmaniasis. A total of 80,000 pfu of an amplified *L. chagasi* genomic library were screened with the entire coding region of LmgSP9 (amplified from *L. major* genomic DNA). Seven hybridizing clones were purified to homogeneity. The determined DNA sequences for two of these clones, referred to as Lc Gene A and LcGene B, are provided in SEQ ID NO: 59 and 60, respectively, with the corresponding predicted amino acid sequences being provided in SEQ ID NO: 61 and 62, respectively. The open reading frame for Lc Gene A was found to show some homology to Gene A/C, previously isolated from *L. major* (McKlean et al., *Mol. Bio. Parasitol.*, 85:221-231, 1997). The open reading frame for Lc Gene B showed some homology to Gene B of *L. major*, discussed above, and was found to contain eleven repeats of a 14 amino acid repeat unit (SEQ ID NO: 63), with each repeat being further divided into two 7 amino acid units, provided in SEQ ID NO: 64 and 65.

The diagnostic potentials of Lc Gene A and Lc Gene B were evaluated by ELISA as described above using sera from visceral leishmaniasis patients from Sudan and Brazil, and from uninfected controls. Absorbance values were compared to those obtained using LmgSP9. Much higher absorbance values were obtained with Lc Gene A and Lc Gene B than with LmgSP9, with Lc Gene B appearing to be more effective than Lc Gene A in detecting antibodies in certain cases. These results indicate that Lc Gene B is highly effective in the diagnosis of visceral leishmaniasis.

In order to assess the diagnostic potential of the repeats found within Lc Gene B, a series of 6 peptides were synthesized (SEQ ID NO: 66-71; referred to as Pep 1-6), differing in an R or H residue. An ELISA was carried out using the full-length LcGene B protein and the six peptides. The absorbance values obtained with Pep 3 were higher than those obtained with the other 5 peptides, however they were not as high as those obtained with the full length protein.

EXAMPLE 14ISOLATION AND CHARACTERIZATION OF DNA ENCODING FOR SOLUBLE ANTIGENS FROM
AN *L. CHAGASI* GENOMIC DNA LIBRARY

5 This example illustrates the preparation of five soluble *Leishmania* antigen genes from an *L. chagasi* genomic DNA library.

 An *L. chagasi* genomic DNA expression library was prepared from *L. chagasi* promastigotes using the unidirectional Lambda ZAP (uni-ZAP) kit (Stratagene) according to the manufacturer's protocol. This library was screened with a high titer
10 rabbit sera raised against *L. major* soluble antigens, as described above in Example 9. Five positive clones were identified. The phagemid were excised and DNA from each of the Five clones was sequenced using a Perkin Elmer/Applied Biosystems Division automated sequencer Model 373A. The DNA sequences for these antigens, referred to as LcgSP1, LcgSP3, LcgSP4, LcgSP8, and LcgSP10 are provided in SEQ ID NO:44-
15 48, respectively, with the corresponding amino acid sequences being provided in SEQ ID NO:49-53, respectively.

 Comparison of these sequences with known sequences in the gene bank as described above, revealed no known homologies to LcgSP3, LcgSP4, LcgSP8 and LcgSP10. LcgSP1 was found to be homologous to the known antigen HSP70.

20 Figures 30A and B illustrate the proliferative response of murine lymph nodes to recombinant LcgSP8, LcgSP10 and LcgSP3. Lymph nodes were taken BALB/c mice 17 days after infection with *L. major*. Infection occurred by footpad injection of 2×10^6 parasites/footpad. The cells were stimulated with recombinant antigen and proliferation was measured at 72 hours using ^3H -thymidine. Figure 30A
25 shows the CPM, a direct measurement of mitotic activity in response to the antigens, and Figure 30B shows the stimulation index, which measures the proliferative response relative to the negative control.

EXAMPLE 15ISOLATION OF DNA ENCODING FOR *L. MAJOR* ANTIGENS
BY CD4+ T CELL EXPRESSION CLONING

5

This example illustrates the isolation of T cell antigens of *L. major* using a direct T cell screening approach.

Leishmania-specific CD4+ T cell lines were derived from the PBMC of an individual who tested positive in a leishmania skin test but had no clinical history of
10 disease. These T cell lines were used to screen a *L. major* amastigote cDNA expression library prepared as described in Example 1. Immunoreactive clones were isolated and sequenced as described above. The determined cDNA sequences for the 8 isolated clones, referred to as 1G6-34, 1E6-44, 4A5-63, 1B11-39, 2A10-37, 4G2-83, 4H6-41, 8G3-100 are provided in SEQ ID NO: 72-79, respectively, with the corresponding
15 predicted amino acid sequences being provided in SEQ ID NO: 80-87, respectively. The cDNA sequences provided for 1E6-44, 2A10-37, 4G2-83, 4H6-41 and 8G3-100 are believed to represent partial clones. All of these clones were shown to stimulate T cell proliferation.

Comparison of these sequences with those in the gene bank as described
20 above revealed no known homologies to the antigen 4A5-63. 1G6-34 was found to have some homology to histone H2B previously identified in *L. enrietti*. Antigens 1E6-44, 1B11-39 and 8G3-100 showed some homology to sequences previously identified in other eukaryotes, in particular *Saccharomyces cerevisiae*. 2A10-37 and 4H6-41 were found to be homologous to the two previously identified proteins alpha tubulin from *L.*
25 *donovani* and beta tubulin from *L. major*, respectively, and 4G2-83 was found to be homologous to elongation initiation factor 2 previously identified in *T. cruzi*.

EXAMPLE 15

SYNTHESIS OF POLYPEPTIDES

Polypeptides may be synthesized on a Perkin Elmer/Applied Biosystems
5 Division 430A peptide synthesizer using Fmoc chemistry with HPTU (O-Benzotriazole-N,N,N',N'-tetramethyluronium hexafluorophosphate) activation. A Gly-Cys-Gly sequence may be attached to the amino terminus of the peptide to provide a method of conjugation, binding to an immobilized surface, or labeling of the peptide. Cleavage of the peptides from the solid support may be carried out using the following
10 cleavage mixture: trifluoroacetic acid:ethanedithiol:thioanisole:water:phenol (40:1:2:2:3). After cleaving for 2 hours, the peptides may be precipitated in cold methyl-t-butyl-ether. The peptide pellets may then be dissolved in water containing 0.1% trifluoroacetic acid (TFA) and lyophilized prior to purification by C18 reverse phase HPLC. A gradient of 0%-60% acetonitrile (containing 0.1% TFA) in water
15 (containing 0.1% TFA) may be used to elute the peptides. Following lyophilization of the pure fractions, the peptides may be characterized using electrospray or other types of mass spectrometry and by amino acid analysis.

20 From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for the purpose of illustration, various modifications may be made without deviating from the spirit and scope of the invention.

65

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Corixa Corporation
- (ii) TITLE OF INVENTION: LEISHMANIA ANTIGENS FOR USEIN THE THERAPY AND
DIAGNOSIS OF LEISHMANIASIS
- (iii) NUMBER OF SEQUENCES: 87
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: SEED and BERRY LLP
 - (B) STREET: 6300 Columbia Center, 701 Fifth Avenue
 - (C) CITY: Seattle
 - (D) STATE: Washington
 - (E) COUNTRY: USA
 - (F) ZIP: 98104-7092
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: US
 - (B) FILING DATE: 12-FEB-1998
 - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Maki, David J.
 - (B) REGISTRATION NUMBER: 31,392
 - (C) REFERENCE/DOCKET NUMBER: 210121.42001PC
- (ix) TELECOMMUNICATION INFORMATION:
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(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 3134 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 421..2058

(xi) SEQUENCE DESCRIPTION: SkeQ ID NO:1:

CAAGTGTCGA AGGACAGTGT TCNCCGTGTG AGATCGCCGG CTGTGCGTGT GAAGGCGGTG	60
CCATCGGANA AACAAACACCG GTGGANCCGC AGGAAACCAT CTTTCTCCGC AGGTCTCTTT	120
TTGTTGTGCGA TTGAGAGTGC NCCAAACCCT GCTGGTGCCC TTCTCACATA TCATGTTTTT	180
CGTTGTGCGC TCGCTTTGCC TTTCTCTCC TTTCCCTCTC TTCCGTGGTG CCGTGTATAC	240
TTCTGGCACC CGCTACGTCA CTTGCTGGT TTGAACAGAA CCACTGTGAA CACCCACGGG	300
CGATCGCACA CATAACATC CCTCACTCAC ACACACAGCT ACATCTATCC TACATAAAGC	360
TGAAAAAAAA GTCTACGAAC AATTTTGT TTACAGTGCG TTGCCGCACA TTTCTCCGTA	420
ATG GAC GCA ACT GAG CTG AAG AAC AAG GGG AAC GAA GAG TTC TCC GCC	468
Met Asp Ala Thr Glu Leu Lys Asn Lys Gly Asn Glu Glu Phe Ser Ala	
1 5 10 15	
GGC CGC TAT GTG GAG GCG GTG AAC TAC TTC TCA AAG GCG ATC CAG TTG	516
Gly Arg Tyr Val Glu Ala Val Asn Tyr Phe Ser Lys Ala Ile Gln Leu	
20 25 30	
GAT GAG CAG AAC AGT GTC CTC TAC AGC AAC CGC TCC GCC TGT TTT GCA	564
Asp Glu Gln Asn Ser Val Leu Tyr Ser Asn Arg Ser Ala Cys Phe Ala	
35 40 45	
GCC ATG CAG AAA TAC AAG GAC GCG CTG GAC GAC GCC GAC AAG TGC ATC	612
Ala Met Gln Lys Tyr Lys Asp Ala Leu Asp Asp Ala Asp Lys Cys Ile	
50 55 60	
TCG ATC AAG CCG AAT TGG GCC AAG GGC TAC GTG CGC CGA GGA GCA GCT	660
Ser Ile Lys Pro Asn Trp Ala Lys Gly Tyr Val Arg Arg Gly Ala Ala	
65 70 75 80	
CTC CAT GGC ATG CGC CGC TAC GAC GAT GCC ATT GCC GCG TAT GAA AAG	708
Leu His Gly Met Arg Arg Tyr Asp Asp Ala Ile Ala Ala Tyr Glu Lys	
85 90 95	
GGG CTC AAG GTG GAC CCT TCC AAC AGC GGC TGC GCG CAG GGC GTG AAG	756
Gly Leu Lys Val Asp Pro Ser Asn Ser Gly Cys Ala Gln Gly Val Lys	
100 105 110	
GAC GTG CAG GTA GCC AAG GCC CGC GAA GCA CGT GAC CCC ATC GCT CGC	804
Asp Val Gln Val Ala Lys Ala Arg Glu Ala Arg Asp Pro Ile Ala Arg	
115 120 125	
GTC TTC ACC CCG GAG GCG TTC CGC AAG ATC CAA GAG AAT CCC AAG CTG	852
Val Phe Thr Pro Glu Ala Phe Arg Lys Ile Gln Glu Asn Pro Lys Leu	
130 135 140	
TCT CTA CTT ATG CTG CAG CCG GAC TAC GTG AAG ATG GTA GAC ACC GTC	900
Ser Leu Leu Met Leu Gln Pro Asp Tyr Val Lys Met Val Asp Thr Val	

67

145	150	155	160	
ATC CGC GAC CCT TCG CAG GGC CGG CTG TAC ATG GAA GAC CAG CGC TTT				948
Ile Arg Asp Pro Ser Gln Gly Arg Leu Tyr Met Glu Asp Gln Arg Phe	165	170	175	
GCC CTG ACG CTC ATG TAC CTG AGC GGA ATG AAG ATT CCC AAC GAT GGT				996
Ala Leu Thr Leu Met Tyr Leu Ser Gly Met Lys Ile Pro Asn Asp Gly	180	185	190	
GAT GGC GAG GAG GAG GAA CGT CCG TCT GCG AAG GCG GCA GAG ACA GCG				1044
Asp Gly Glu Glu Glu Glu Arg Pro Ser Ala Lys Ala Ala Glu Thr Ala	195	200	205	
AAG CCA AAA GAG GAG AAG CCT CTC ACC GAC AAC GAG AAG GAG GCC CTG				1092
Lys Pro Lys Glu Glu Lys Pro Leu Thr Asp Asn Glu Lys Glu Ala Leu	210	215	220	
GCG CTC AAG GAG GAG GGC AAC AAG CTG TAC CTC TCG AAG AAG TTT GAG				1140
Ala Leu Lys Glu Glu Gly Asn Lys Leu Tyr Leu Ser Lys Lys Phe Glu	225	230	235	240
GAG GCG CTG ACC AAG TAC CAA GAG GCG CAG GTG AAA GAC CCC AAC AAC				1188
Glu Ala Leu Thr Lys Tyr Gln Glu Ala Gln Val Lys Asp Pro Asn Asn	245	250	255	
ACT TTA TAC ATT CTG AAC GTG TCG GCC GTG TAC TTC GAG CAG GGT GAC				1236
Thr Leu Tyr Ile Leu Asn Val Ser Ala Val Tyr Phe Glu Gln Gly Asp	260	265	270	
TAC GAC AAG TGC ATC GCC GAG TGC GAG CAC GGT ATC GAG CAC GGT CGC				1284
Tyr Asp Lys Cys Ile Ala Glu Cys Glu His Gly Ile Glu His Gly Arg	275	280	285	
GAG AAC CAC TGC GAC TAC ACA ATC ATT GCG AAG CTC ATG ACC CGG AAC				1332
Glu Asn His Cys Asp Tyr Thr Ile Ile Ala Lys Leu Met Thr Arg Asn	290	295	300	
GCC TTG TGC CTC CAG AGG CAG AGG AAG TAC GAG GCT GCT ATC GAC CTT				1380
Ala Leu Cys Leu Gln Arg Gln Arg Lys Tyr Glu Ala Ala Ile Asp Leu	305	310	315	320
TAC AAG CGC GCC CTT GTC GAG TGG CGT AAC CCT GAC ACC CTC AAG AAG				1428
Tyr Lys Arg Ala Leu Val Glu Trp Arg Asn Pro Asp Thr Leu Lys Lys	325	330	335	
CTG ACG GAG TGC GAG AAG GAG CAC CAA AAG GCG GTG GAG GAA GCC TAC				1476
Leu Thr Glu Cys Glu Lys Glu His Gln Lys Ala Val Glu Glu Ala Tyr	340	345	350	
ATC GAT CCT GAG ATC GCG AAG CAG AAG AAA GAC GAA GGT AAC CAG TAC				1524
Ile Asp Pro Glu Ile Ala Lys Gln Lys Lys Asp Glu Gly Asn Gln Tyr	355	360	365	
TTC AAG GAG GAT AAG TTC CCC GAG GCC GTG GCA GCG TAC ACG GAG GCC				1572

68

Phe Lys Glu Asp Lys Phe Pro Glu Ala Val Ala Ala Tyr Thr Glu Ala	
370 375 380	
ATC AAG CGC AAC CCT GCC GAG CAC ACC TCC TAC AGC AAT CGC GCG GCC	1620
Ile Lys Arg Asn Pro Ala Glu His Thr Ser Tyr Ser Asn Arg Ala Ala	
385 390 395 400	
GCG TAC ATC AAG CTT GGA GCC TTC AAC GAC GCC CTC AAG GAC GCG GAG	1668
Ala Tyr Ile Lys Leu Gly Ala Phe Asn Asp Ala Leu Lys Asp Ala Glu	
405 410 415	
AAG TGC ATT GAG CTG AAG CCC GAC TTT GTT AAG GGC TAC GCG CGC AAG	1716
Lys Cys Ile Glu Leu Lys Pro Asp Phe Val Lys Gly Tyr Ala Arg Lys	
420 425 430	
GGT CAT GCT TAC TTT TGG ACC AAG CAG TAC AAC CGC GCG CTG CAG GCG	1764
Gly His Ala Tyr Phe Trp Thr Lys Gln Tyr Asn Arg Ala Leu Gln Ala	
435 440 445	
TAC GAT GAG GGC CTC AAG GTG GAC CCG AGC AAT GCG GAC TGC AAG GAT	1812
Tyr Asp Glu Gly Leu Lys Val Asp Pro Ser Asn Ala Asp Cys Lys Asp	
450 455 460	
GGG CGG TAT CGC ACA ATC ATG AAG ATT CAG GAG ATG GCA TCT GGC CAA	1860
Gly Arg Tyr Arg Thr Ile Met Lys Ile Gln Glu Met Ala Ser Gly Gln	
465 470 475 480	
TCC GCG GAT GGC GAC GAG GCG GCG CGC CGG GCC ATG GAC GAT CCT GAA	1908
Ser Ala Asp Gly Asp Glu Ala Ala Arg Arg Ala Met Asp Asp Pro Glu	
485 490 495	
ATC GCG GCA ATC ATG CAA GAT AGC TAC ATG CAA CTA GTG TTG AAG GAG	1956
Ile Ala Ala Ile Met Gln Asp Ser Tyr Met Gln Leu Val Leu Lys Glu	
500 505 510	
ATG CAG AAC GAT CCC ACG CGC ATT CAG GAG TAC ATG AAG GAC TCC GGG	2004
Met Gln Asn Asp Pro Thr Arg Ile Gln Glu Tyr Met Lys Asp Ser Gly	
515 520 525	
ATC TCA TCG AAG ATC AAC AAG CTG ATT TCA GCT GGC ATC ATT CGT TTT	2052
Ile Ser Ser Lys Ile Asn Lys Leu Ile Ser Ala Gly Ile Ile Arg Phe	
530 535 540	
GGT CAG TAGACTTCTA CGCTGCCTCA TCTTTTCCGT GTCTTTGCGT CGGCGGGTAT	2108
Gly Gln	
545	
CGTAAAGCAC AATAAAGCAG CGATTACAT GCACGAGTAA AGTGCTGCGC CTCTCAAACA	2168
CGACGTCGAG GCTGTGGTGC AGATGCGCGT CCTGCATGAA GGTAGTGAAG AGGAAAGTAA	2228
GGGATGTTGT TTGTGGGCCT TCGTGGCTGC GCACACACCT CTTATCTCCT TCGCTTGGTA	2288
CCTTCTCCCT TTTTCGTCTT CACCCCCCTT TCTCTTCTCA CGCTCTCCCT GGCGCGGTGG	2348

69

TGCAACGATT TCGTTTTATT TACGTCTGTG TAGCTCCTCT ATTCAACGGT GCGATGACGC	2408
TAACGAAGCT GGCCTGTATT CGGCTAAGGC GAAGGCAAAA GACTAGGAGG GGGGGGGGAA	2468
GGAGACGGCG TGACCATCAC TGCGAAGAAA CAAGCCGAAG AAAAGGCCCC GAACGCCTGC	2528
ATTTCCGCGC GCCCTCGCCC GCCTTCCTTC CTTCTTCGCG TCTCTCTCTC TCTCTCTCTC	2588
GCTATCTTCT CAACGGAGAC ATGAAAGGCG TTTGTTAGGA AAAGAGGGGG GGGGGAAGAG	2648
TGGGACGACG CGCTGCGTCT TTTGGGCACT GGTCACGTGC GTCACCCTCT TTTTTATCT	2708
CTATTGGCAC TGTCTTGTTT CTTTTCCCTT TCCTATCATA CGCGTCTCGC AAACGACTCC	2768
GCGCTGAGCA GCCATGTGCT GCGGCGTGGA GGAAGTACAC AGACATCACG GATGCATATG	2828
TGCGCGTCCG TGTACGCGCT TGTATGGGGC TTCTAACAGC GCCTGTGTGT GTTTGTGTGT	2888
GTGTGTGTGT GTGTGTCTGT GTATTTGAG CGTCTGTATG CTATTCTATT AAGCACCGAA	2948
GAAGAGACAC ACACGACAGC GAAGGAGATG GTGTCGGCTT TTCGGCTAAT CACTCCCTTC	3008
CATAGCTTCT CTGAAGGAGG CTCTCTTCCA GAGGAATAGA CTGCAGATGG GGTCCACGTT	3068
TATCTGAGGA GTCAACGGAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA	3128
CTCGAG	3134

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 546 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met	Asp	Ala	Thr	Glu	Leu	Lys	Asn	Lys	Gly	Asn	Glu	Glu	Phe	Ser	Ala
1				5					10					15	
Gly	Arg	Tyr	Val	Glu	Ala	Val	Asn	Tyr	Phe	Ser	Lys	Ala	Ile	Gln	Leu
			20					25					30		
Asp	Glu	Gln	Asn	Ser	Val	Leu	Tyr	Ser	Asn	Arg	Ser	Ala	Cys	Phe	Ala
		35				40						45			
Ala	Met	Gln	Lys	Tyr	Lys	Asp	Ala	Leu	Asp	Asp	Ala	Asp	Lys	Cys	Ile
	50					55					60				
Ser	Ile	Lys	Pro	Asn	Trp	Ala	Lys	Gly	Tyr	Val	Arg	Arg	Gly	Ala	Ala
	65				70					75				80	

Leu His Gly Met Arg Arg Tyr Asp Asp Ala Ile Ala Ala Tyr Glu Lys
85 90 95

Gly Leu Lys Val Asp Pro Ser Asn Ser Gly Cys Ala Gln Gly Val Lys
100 105 110

Asp Val Gln Val Ala Lys Ala Arg Glu Ala Arg Asp Pro Ile Ala Arg
115 120 125

Val Phe Thr Pro Glu Ala Phe Arg Lys Ile Gln Glu Asn Pro Lys Leu
130 135 140

Ser Leu Leu Met Leu Gln Pro Asp Tyr Val Lys Met Val Asp Thr Val
145 150 155 160

Ile Arg Asp Pro Ser Gln Gly Arg Leu Tyr Met Glu Asp Gln Arg Phe
165 170 175

Ala Leu Thr Leu Met Tyr Leu Ser Gly Met Lys Ile Pro Asn Asp Gly
180 185 190

Asp Gly Glu Glu Glu Glu Arg Pro Ser Ala Lys Ala Ala Glu Thr Ala
195 200 205

Lys Pro Lys Glu Glu Lys Pro Leu Thr Asp Asn Glu Lys Glu Ala Leu
210 215 220

Ala Leu Lys Glu Glu Gly Asn Lys Leu Tyr Leu Ser Lys Lys Phe Glu
225 230 235 240

Glu Ala Leu Thr Lys Tyr Gln Glu Ala Gln Val Lys Asp Pro Asn Asn
245 250 255

Thr Leu Tyr Ile Leu Asn Val Ser Ala Val Tyr Phe Glu Gln Gly Asp
260 265 270

Tyr Asp Lys Cys Ile Ala Glu Cys Glu His Gly Ile Glu His Gly Arg
275 280 285

Glu Asn His Cys Asp Tyr Thr Ile Ile Ala Lys Leu Met Thr Arg Asn
290 295 300

Ala Leu Cys Leu Gln Arg Gln Arg Lys Tyr Glu Ala Ala Ile Asp Leu
305 310 315 320

Tyr Lys Arg Ala Leu Val Glu Trp Arg Asn Pro Asp Thr Leu Lys Lys
325 330 335

Leu Thr Glu Cys Glu Lys Glu His Gln Lys Ala Val Glu Glu Ala Tyr
340 345 350

Ile Asp Pro Glu Ile Ala Lys Gln Lys Lys Asp Glu Gly Asn Gln Tyr
355 360 365

Phe Lys Glu Asp Lys Phe Pro Glu Ala Val Ala Ala Tyr Thr Glu Ala

71

370	375	380
Ile Lys Arg Asn Pro Ala Glu His Thr Ser Tyr Ser Asn Arg Ala Ala		
385	390	395 400
Ala Tyr Ile Lys Leu Gly Ala Phe Asn Asp Ala Leu Lys Asp Ala Glu		
	405	410 415
Lys Cys Ile Glu Leu Lys Pro Asp Phe Val Lys Gly Tyr Ala Arg Lys		
	420	425 430
Gly His Ala Tyr Phe Trp Thr Lys Gln Tyr Asn Arg Ala Leu Gln Ala		
	435	440 445
Tyr Asp Glu Gly Leu Lys Val Asp Pro Ser Asn Ala Asp Cys Lys Asp		
	450	455 460
Gly Arg Tyr Arg Thr Ile Met Lys Ile Gln Glu Met Ala Ser Gly Gln		
	465	470 475 480
Ser Ala Asp Gly Asp Glu Ala Ala Arg Arg Ala Met Asp Asp Pro Glu		
	485	490 495
Ile Ala Ala Ile Met Gln Asp Ser Tyr Met Gln Leu Val Leu Lys Glu		
	500	505 510
Met Gln Asn Asp Pro Thr Arg Ile Gln Glu Tyr Met Lys Asp Ser Gly		
	515	520 525
Ile Ser Ser Lys Ile Asn Lys Leu Ile Ser Ala Gly Ile Ile Arg Phe		
	530	535 540
Gly Gln		
545		

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 676 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 26..550

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

AATTCGGCAC GAGGCATTGT GCATA ATG GTC AAG TCC CAC TAC ATC TGC GCG
 Met Val Lys Ser His Tyr Ile Cys Ala
 550 555

72

GGC CGC CTG GTG CGC ATC CTG CGT GGC CCC CGC CAG GAC CGC GTT GGT	100
Gly Arg Leu Val Arg Ile Leu Arg Gly Pro Arg Gln Asp Arg Val Gly	
560 565 570	
GTG ATC GTC GAC ATT GTC GAC GCG AAC CGC GTG CTG GTG GAG AAC CCG	148
Val Ile Val Asp Ile Val Asp Ala Asn Arg Val Leu Val Glu Asn Pro	
575 580 585	
GAG GAC GCG AAG ATG TGG CGC CAC GTG CAG AAC CTG AAG AAC GTG GAG	196
Glu Asp Ala Lys Met Trp Arg His Val Gln Asn Leu Lys Asn Val Glu	
590 595 600	
CCG CTG AAG TAC TGC GTG AGC GTC AGC CGC AAC TGC AGC GCG AAG GCG	244
Pro Leu Lys Tyr Cys Val Ser Val Ser Arg Asn Cys Ser Ala Lys Ala	
605 610 615	
CTG AAG GAT GCG CTG GCC TCG TCG AAG GCG CTG GAG AAG TAC GCG AAG	292
Leu Lys Asp Ala Leu Ala Ser Ser Lys Ala Leu Glu Lys Tyr Ala Lys	
620 625 630 635	
ACG CGC ACT GCT GCG CGC GTG GAG GCG AAG AAG GCG TGC GCC GCG TCG	340
Thr Arg Thr Ala Ala Arg Val Glu Ala Lys Lys Ala Cys Ala Ala Ser	
640 645 650	
ACG GAC TTC GAG CGC TAC CAG CTG CGC GTT GCG CGC CGT TCT CGC GCG	388
Thr Asp Phe Glu Arg Tyr Gln Leu Arg Val Ala Arg Arg Ser Arg Ala	
655 660 665	
CAC TGG GCG CGC AAG GTG TTC GAC GAG AAG GAC GCG AAG ACG CCC GTG	436
His Trp Ala Arg Lys Val Phe Asp Glu Lys Asp Ala Lys Thr Pro Val	
670 675 680	
TCG TGG CAC AAG GTT GCG CTG AAG AAG ATG CAG AAG AAG GCC GCA AAG	484
Ser Trp His Lys Val Ala Leu Lys Lys Met Gln Lys Lys Ala Ala Lys	
685 690 695	
ATG GAC TCG ACC GAG GGC GCT AAG AGG CGC ATG CAG AAG GCG ATC GCT	532
Met Asp Ser Thr Glu Gly Ala Lys Arg Arg Met Gln Lys Ala Ile Ala	
700 705 710 715	
GCC CGC AAG GCG AAA AAG TAAGGCCATA CCCTCACTTC GCTTGTTTCG	580
Ala Arg Lys Ala Lys Lys	
720	
TGATTTTTCG TGGGAGTCGG TGGCCCTACC AGCGGTCTTT CATTGGCTTA TTTCTATCCG	640
GTCTGAAAGA GGTACAAAAA AAAAAAAAAA AAAAAA	676

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 175 amino acids

(B) TYPE: amino acid

73

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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Met Val Lys Ser His Tyr Ile Cys Ala Gly Arg Leu Val Arg Ile Leu
 1             5             10             15

Arg Gly Pro Arg Gln Asp Arg Val Gly Val Ile Val Asp Ile Val Asp
      20             25             30

Ala Asn Arg Val Leu Val Glu Asn Pro Glu Asp Ala Lys Met Trp Arg
      35             40             45

His Val Gln Asn Leu Lys Asn Val Glu Pro Leu Lys Tyr Cys Val Ser
      50             55             60

Val Ser Arg Asn Cys Ser Ala Lys Ala Leu Lys Asp Ala Leu Ala Ser
      65             70             75             80

Ser Lys Ala Leu Glu Lys Tyr Ala Lys Thr Arg Thr Ala Ala Arg Val
      85             90             95

Glu Ala Lys Lys Ala Cys Ala Ala Ser Thr Asp Phe Glu Arg Tyr Gln
      100            105            110

Leu Arg Val Ala Arg Arg Ser Arg Ala His Trp Ala Arg Lys Val Phe
      115            120            125

Asp Glu Lys Asp Ala Lys Thr Pro Val Ser Trp His Lys Val Ala Leu
      130            135            140

Lys Lys Met Gln Lys Lys Ala Ala Lys Met Asp Ser Thr Glu Gly Ala
      145            150            155            160

Lys Arg Arg Met Gln Lys Ala Ile Ala Ala Arg Lys Ala Lys Lys
      165            170            175

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(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2040 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 62..2029

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CGCGGTGGCG GCCGCTCTAG AACTAGTGGG TCCCCCGGGC TGCAGGAATT CGGCACGAGA	60
G AGC CTG ACG GAC CCG GCG GTG CTG GGC GAG GAG ACT CAC CTG CGC	106
Ser Leu Thr Asp Pro Ala Val Leu Gly Glu Glu Thr His Leu Arg	
180 185 190	
GTC CGC GTG GTG CCG GAC AAG GCG AAC AAG ACG CTG ACG GTG GAG GAT	154
Val Arg Val Val Pro Asp Lys Ala Asn Lys Thr Leu Thr Val Glu Asp	
195 200 205	
AAC GGC ATC GGC ATG ACC AAG GCG GAC CTC GTG AAC AAT CTG GGC ACG	202
Asn Gly Ile Gly Met Thr Lys Ala Asp Leu Val Asn Asn Leu Gly Thr	
210 215 220	
ATC GCG CGC TCC GGC ACG AAG GCT TTC ATG GAG GCA CTG GAG GCC GGC	250
Ile Ala Arg Ser Gly Thr Lys Ala Phe Met Glu Ala Leu Glu Ala Gly	
225 230 235	
GGC GAC ATG AGC ATG ATC GGC CAG TTC GGT GTC GGC TTC TAC TCC GCG	298
Gly Asp Met Ser Met Ile Gly Gln Phe Gly Val Gly Phe Tyr Ser Ala	
240 245 250	
TAC CTT GTG GCG GAC CGC GTG ACG GTG GTG TCG AAG AAC AAC TCG GAC	346
Tyr Leu Val Ala Asp Arg Val Thr Val Val Ser Lys Asn Asn Ser Asp	
255 260 265 270	
GAG GCG TAC TGG GAA TCG TCT GCG GGG GGC ACG TTC ACC ATC ACG AGC	394
Glu Ala Tyr Trp Glu Ser Ser Ala Gly Gly Thr Phe Thr Ile Thr Ser	
275 280 285	
GTG CAG GAG TCG GAC ATG AAG CGC GGC ACG AGT ACA ACG CTG CAC CTA	442
Val Gln Glu Ser Asp Met Lys Arg Gly Thr Ser Thr Thr Leu His Leu	
290 295 300	
AAG GAG GAC CAG CAG GAG TAC CTG GAG GAG CGC CGG GTG AAG GAG CTG	490
Lys Glu Asp Gln Gln Glu Tyr Leu Glu Glu Arg Arg Val Lys Glu Leu	
305 310 315	
ATC AAG AAG CAC TCC GAG TTC ATC GGC TAC GAC ATC GAG CTG ATG GTG	538
Ile Lys Lys His Ser Glu Phe Ile Gly Tyr Asp Ile Glu Leu Met Val	
320 325 330	
GAG AAG ACG GCG GAG AAG GAG GTG ACG GAC GAG GAC GAG GAG GAG GAC	586
Glu Lys Thr Ala Glu Lys Glu Val Thr Asp Glu Asp Glu Glu Glu Asp	
335 340 345 350	
GAG TCG AAG AAG AAG TCC TGC GGG GAC GAG GGC GAG CCG AAG GTG GAG	634
Glu Ser Lys Lys Lys Ser Cys Gly Asp Glu Gly Glu Pro Lys Val Glu	
355 360 365	
GAG GTG ACG GAG GGC GGC GAG GAC AAG AAG AAG AAG ACG AAG AAG GTG	682
Glu Val Thr Glu Gly Gly Glu Asp Lys Lys Lys Lys Thr Lys Lys Val	
370 375 380	

75

AAG GAG GTG AAG AAG ACG TAC GAG GTC AAG AAC AAG CAC AAG CCG CTC	730
Lys Glu Val Lys Lys Thr Tyr Glu Val Lys Asn Lys His Lys Pro Leu	
385 390 395	
TGG ACG CGC GAC ACG AAG GAC GTG ACG AAG GAG GAG TAC GCG GCC TTC	778
Trp Thr Arg Asp Thr Lys Asp Val Thr Lys Glu Glu Tyr Ala Ala Phe	
400 405 410	
TAC AAG GCC ATC TCC AAC GAC TGG GAG GAC ACG GCG GCG ACG AAG CAC	826
Tyr Lys Ala Ile Ser Asn Asp Trp Glu Asp Thr Ala Ala Thr Lys His	
415 420 425 430	
TTC TCG GTG GAG GGC CAG CTG GAG TTC CGC GCG ATC GCG TTC GTG CCG	874
Phe Ser Val Glu Gly Gln Leu Glu Phe Arg Ala Ile Ala Phe Val Pro	
435 440 445	
AAG CGC GCG CCG TTC GAC ATG TTC GAG CCG AAC AAG AAG CGC AAC AAC	922
Lys Arg Ala Pro Phe Asp Met Phe Glu Pro Asn Lys Lys Arg Asn Asn	
450 455 460	
ATC AAG CTG TAC GTG CGC CGC GTG TTC ATC ATG GAC AAC TGC GAG GAC	970
Ile Lys Leu Tyr Val Arg Arg Val Phe Ile Met Asp Asn Cys Glu Asp	
465 470 475	
CTG TGC CCG GAC TGG CTC GGC TTC GTG AAG GGC GTC GTG GAC AGC GAG	1018
Leu Cys Pro Asp Trp Leu Gly Phe Val Lys Gly Val Val Asp Ser Glu	
480 485 490	
GAC CTG CCG CTG AAC ATC TCG CGC GAG AAC CTG CAG CAG AAC AAG ATC	1066
Asp Leu Pro Leu Asn Ile Ser Arg Glu Asn Leu Gln Gln Asn Lys Ile	
495 500 505 510	
CTG AAG GTG ATC CGC AAG AAC ATC GTG AAG AAG TGC CTG GAG CTG TTC	1114
Leu Lys Val Ile Arg Lys Asn Ile Val Lys Lys Cys Leu Glu Leu Phe	
515 520 525	
GAA GAG ATA GCG GAG AAC AAG GAG GAC TAC AAG CAG TTC TAC GAG CAG	1162
Glu Glu Ile Ala Glu Asn Lys Glu Asp Tyr Lys Gln Phe Tyr Glu Gln	
530 535 540	
TTC GGC AAG AAC ATC AAG CTG GGC ATC CAC GAG GAC ACG GCG AAC CGC	1210
Phe Gly Lys Asn Ile Lys Leu Gly Ile His Glu Asp Thr Ala Asn Arg	
545 550 555	
AAG AAG CTG ATG GAG TTG CTG CGC TTC TAC AGC ACC GAG TCG GGG GAG	1258
Lys Lys Leu Met Glu Leu Leu Arg Phe Tyr Ser Thr Glu Ser Gly Glu	
560 565 570	
GAG ATG ACG ACA CTG AAG GAC TAC GTG ACG CGC ATG AAG CCG GAG CAG	1306
Glu Met Thr Thr Leu Lys Asp Tyr Val Thr Arg Met Lys Pro Glu Gln	
575 580 585 590	
AAG TCG ATC TAC TAC ATC ACT GGC GAC AGC AAG AAG AAG CTG GAG TCG	1354
Lys Ser Ile Tyr Tyr Ile Thr Gly Asp Ser Lys Lys Lys Leu Glu Ser	
595 600 605	

TCG CCG TTC ATC GAG AAG GCG AGA CGC TGC GGG CTC GAG GTG CTG TTC	1402
Ser Pro Phe Ile Glu Lys Ala Arg Arg Cys Gly Leu Glu Val Leu Phe	
610 615 620	
ATG ACG GAG CCG ATC GAC GAG TAC GTG ATG CAG CAG GTG AAG GAC TTC	1450
Met Thr Glu Pro Ile Asp Glu Tyr Val Met Gln Gln Val Lys Asp Phe	
625 630 635	
GAG GAC AAG AAG TTC GCG TGC CTG ACG AAG GAA GGC GTG CAC TTC GAG	1498
Glu Asp Lys Lys Phe Ala Cys Leu Thr Lys Glu Gly Val His Phe Glu	
640 645 650	
GAG TCC GAG GAG GAG AAG AAG CAG CGC GAG GAG AAG AAG GCG GCG TGC	1546
Glu Ser Glu Glu Glu Lys Lys Gln Arg Glu Glu Lys Lys Ala Ala Cys	
655 660 665 670	
GAG AAG CTG TGC AAG ACG ATG AAG GAG GTG CTG GGC GAC AAG GTG GAG	1594
Glu Lys Leu Cys Lys Thr Met Lys Glu Val Leu Gly Asp Lys Val Glu	
675 680 685	
AAG GTG ACC GTG TCG GAG CGC CTG TTG ACG TCG CCG TGC ATC CTG GTG	1642
Lys Val Thr Val Ser Glu Arg Leu Leu Thr Ser Pro Cys Ile Leu Val	
690 695 700	
ACG TCG GAG TTT GGG TGG TCG GCG CAC ATG GAA CAG ATC ATG CGC AAC	1690
Thr Ser Glu Phe Gly Trp Ser Ala His Met Glu Gln Ile Met Arg Asn	
705 710 715	
CAG GCG CTG CGC GAC TCC AGC ATG GCG CAG TAC ATG GTG TCC AAG AAG	1738
Gln Ala Leu Arg Asp Ser Ser Met Ala Gln Tyr Met Val Ser Lys Lys	
720 725 730	
ACG ATG GAG GTG AAC CCC GAC CAC CCC ATC ATC AAG GAG CTG CGC CGC	1786
Thr Met Glu Val Asn Pro Asp His Pro Ile Ile Lys Glu Leu Arg Arg	
735 740 745 750	
CGC GTG GAG GCG GAC GAG AAC GAC AAG GCC GTG AAG GAC CTC GTC TTC	1834
Arg Val Glu Ala Asp Glu Asn Asp Lys Ala Val Lys Asp Leu Val Phe	
755 760 765	
CTG CTC TTC GAC ACG TCG CTG CTC ACG TCC GGC TTC CAG CTG GAT GAC	1882
Leu Leu Phe Asp Thr Ser Leu Leu Thr Ser Gly Phe Gln Leu Asp Asp	
770 775 780	
CCC ACC GGC TAC GCC GAG CGC ATC AAC CGC ATG ATC AAG CTC GGC CTG	1930
Pro Thr Gly Tyr Ala Glu Arg Ile Asn Arg Met Ile Lys Leu Gly Leu	
785 790 795	
TCG CTC GAC GAG GAG GAG GAG GAG GTC GCC GAG GCG CCG CCG GCC GAG	1978
Ser Leu Asp Glu Glu Glu Glu Glu Val Ala Glu Ala Pro Pro Ala Glu	
800 805 810	
GCA GCC CCC GCG GAG GTC ACC GCC GGC ACC TCC AGC ATG GAG CAG GTG	2026
Ala Ala Pro Ala Glu Val Thr Ala Gly Thr Ser Ser Met Glu Gln Val	

77

815

820

825

830

GAC TGAGCCGGTA A

2040

Asp

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 656 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

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Ser Leu Thr Asp Pro Ala Val Leu Gly Glu Glu Thr His Leu Arg Val
 1           5           10           15
Arg Val Val Pro Asp Lys Ala Asn Lys Thr Leu Thr Val Glu Asp Asn
      20           25           30
Gly Ile Gly Met Thr Lys Ala Asp Leu Val Asn Asn Leu Gly Thr Ile
      35           40           45
Ala Arg Ser Gly Thr Lys Ala Phe Met Glu Ala Leu Glu Ala Gly Gly
      50           55           60
Asp Met Ser Met Ile Gly Gln Phe Gly Val Gly Phe Tyr Ser Ala Tyr
      65           70           75           80
Leu Val Ala Asp Arg Val Thr Val Val Ser Lys Asn Asn Ser Asp Glu
      85           90           95
Ala Tyr Trp Glu Ser Ser Ala Gly Gly Thr Phe Thr Ile Thr Ser Val
      100          105          110
Gln Glu Ser Asp Met Lys Arg Gly Thr Ser Thr Thr Leu His Leu Lys
      115          120          125
Glu Asp Gln Gln Glu Tyr Leu Glu Glu Arg Arg Val Lys Glu Leu Ile
      130          135          140
Lys Lys His Ser Glu Phe Ile Gly Tyr Asp Ile Glu Leu Met Val Glu
      145          150          155          160
Lys Thr Ala Glu Lys Glu Val Thr Asp Glu Asp Glu Glu Glu Asp Glu
      165          170          175
Ser Lys Lys Lys Ser Cys Gly Asp Glu Gly Glu Pro Lys Val Glu Glu
      180          185          190
Val Thr Glu Gly Gly Glu Asp Lys Lys Lys Lys Thr Lys Lys Val Lys

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78

195	200	205
Glu Val Lys Lys Thr Tyr	Glu Val Lys Asn Lys His Lys Pro Leu Trp	
210	215	220
Thr Arg Asp Thr Lys Asp Val Thr Lys Glu Glu Tyr Ala Ala Phe Tyr		
225	230	235 240
Lys Ala Ile Ser Asn Asp Trp Glu Asp Thr Ala Ala Thr Lys His Phe		
245	250	255
Ser Val Glu Gly Gln Leu Glu Phe Arg Ala Ile Ala Phe Val Pro Lys		
260	265	270
Arg Ala Pro Phe Asp Met Phe Glu Pro Asn Lys Lys Arg Asn Asn Ile		
275	280	285
Lys Leu Tyr Val Arg Arg Val Phe Ile Met Asp Asn Cys Glu Asp Leu		
290	295	300
Cys Pro Asp Trp Leu Gly Phe Val Lys Gly Val Val Asp Ser Glu Asp		
305	310	315 320
Leu Pro Leu Asn Ile Ser Arg Glu Asn Leu Gln Gln Asn Lys Ile Leu		
325	330	335
Lys Val Ile Arg Lys Asn Ile Val Lys Lys Cys Leu Glu Leu Phe Glu		
340	345	350
Glu Ile Ala Glu Asn Lys Glu Asp Tyr Lys Gln Phe Tyr Glu Gln Phe		
355	360	365
Gly Lys Asn Ile Lys Leu Gly Ile His Glu Asp Thr Ala Asn Arg Lys		
370	375	380
Lys Leu Met Glu Leu Leu Arg Phe Tyr Ser Thr Glu Ser Gly Glu Glu		
385	390	395 400
Met Thr Thr Leu Lys Asp Tyr Val Thr Arg Met Lys Pro Glu Gln Lys		
405	410	415
Ser Ile Tyr Tyr Ile Thr Gly Asp Ser Lys Lys Lys Leu Glu Ser Ser		
420	425	430
Pro Phe Ile Glu Lys Ala Arg Arg Cys Gly Leu Glu Val Leu Phe Met		
435	440	445
Thr Glu Pro Ile Asp Glu Tyr Val Met Gln Gln Val Lys Asp Phe Glu		
450	455	460
Asp Lys Lys Phe Ala Cys Leu Thr Lys Glu Gly Val His Phe Glu Glu		
465	470	475 480
Ser Glu Glu Glu Lys Lys Gln Arg Glu Glu Lys Lys Ala Ala Cys Glu		
485	490	495

Lys Leu Cys Lys Thr Met Lys Glu Val Leu Gly Asp Lys Val Glu Lys
 500 505 510
 Val Thr Val Ser Glu Arg Leu Leu Thr Ser Pro Cys Ile Leu Val Thr
 515 520 525
 Ser Glu Phe Gly Trp Ser Ala His Met Glu Gln Ile Met Arg Asn Gln
 530 535 540
 Ala Leu Arg Asp Ser Ser Met Ala Gln Tyr Met Val Ser Lys Lys Thr
 545 550 555 560
 Met Glu Val Asn Pro Asp His Pro Ile Ile Lys Glu Leu Arg Arg Arg
 565 570 575
 Val Glu Ala Asp Glu Asn Asp Lys Ala Val Lys Asp Leu Val Phe Leu
 580 585 590
 Leu Phe Asp Thr Ser Leu Leu Thr Ser Gly Phe Gln Leu Asp Asp Pro
 595 600 605
 Thr Gly Tyr Ala Glu Arg Ile Asn Arg Met Ile Lys Leu Gly Leu Ser
 610 615 620
 Leu Asp Glu Glu Glu Glu Glu Val Ala Glu Ala Pro Pro Ala Glu Ala
 625 630 635 640
 Ala Pro Ala Glu Val Thr Ala Gly Thr Ser Ser Met Glu Gln Val Asp
 645 650 655

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1771 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..1698

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CAG GCC CGC GTC CAG GCC CTC GAG GAG GCA GCG CGT CTC CGC GCG GAG	48
Gln Ala Arg Val Gln Ala Leu Glu Glu Ala Ala Arg Leu Arg Ala Glu	
1 5 10 15	
CTG GAG GCG GCC GAG GAG GCG GCC CGC CTG GAT GTC ATG CAT GCG GCC	96
Leu Glu Ala Ala Glu Glu Ala Ala Arg Leu Asp Val Met His Ala Ala	
20 25 30	

80

GAG CAG GCC CGT GTC CAG GCC CTC GAG GAG GCA GCG CGT CTC CGC GCG	144
Glu Gln Ala Arg Val Gln Ala Leu Glu Glu Ala Ala Arg Leu Arg Ala	
35 40 45	
GAG CTG GAG GAG GCC GAG GAG GCG GCC CGC CTG GAT GTC ATG CAT GCG	192
Glu Leu Glu Glu Ala Glu Glu Ala Ala Arg Leu Asp Val Met His Ala	
50 55 60	
GCC GAG CAG GCC CGC GTC CAG GCC CTC GAG GAG GCA GCG CGT CTC CGC	240
Ala Glu Gln Ala Arg Val Gln Ala Leu Glu Glu Ala Ala Arg Leu Arg	
65 70 75 80	
GCG GAG CTG GAG GCT GCC GAG GAG GCG GCG CGC CTG GAG GCC ATG CAC	288
Ala Glu Leu Glu Ala Ala Glu Glu Ala Ala Arg Leu Glu Ala Met His	
85 90 95	
GAG GCC GAG CAG GCC CGC TCC CAG GCC CTC GAG GAG GCA GCG CGT CTC	336
Glu Ala Glu Gln Ala Arg Ser Gln Ala Leu Glu Glu Ala Ala Arg Leu	
100 105 110	
CGC GCG GAG CTG GAG GAA GCC GAG GAG GCG GCC CGC CTG GAT GTC ATG	384
Arg Ala Glu Leu Glu Glu Ala Glu Glu Ala Ala Arg Leu Asp Val Met	
115 120 125	
CAT GCG GCC GAG CAG GCC CGC GTC CAG GCC CTC GAG GAG GCA GCG CGT	432
His Ala Ala Glu Gln Ala Arg Val Gln Ala Leu Glu Glu Ala Ala Arg	
130 135 140	
CTC CGC GCG GAG CTG GAG GAG GCC GAG GAG GCG GCC CGC CTG GAG GCC	480
Leu Arg Ala Glu Leu Glu Glu Ala Glu Glu Ala Ala Arg Leu Glu Ala	
145 150 155 160	
ATG CAC GAG GCC GAG CAG GCC CGC TCC CAG GCC CTC GAG GAG GCA GCG	528
Met His Glu Ala Glu Gln Ala Arg Ser Gln Ala Leu Glu Glu Ala Ala	
165 170 175	
CGT CTC CGC GCG GAG CTG GAG GCG GCC GAG GAG GCG GCC CGC CTG GAT	576
Arg Leu Arg Ala Glu Leu Glu Ala Ala Glu Glu Ala Ala Arg Leu Asp	
180 185 190	
GTC ATG CAC GAG GCC GAG CAG GCC CGT GTC CAG GCC CTC GAG GAG GCG	624
Val Met His Glu Ala Glu Gln Ala Arg Val Gln Ala Leu Glu Glu Ala	
195 200 205	
GCG CGC CTG GAT GTC ATG CAC GAG GCC GAG CAG GCC CGC GTC CAG GCC	672
Ala Arg Leu Asp Val Met His Glu Ala Glu Gln Ala Arg Val Gln Ala	
210 215 220	
CTC GAG GAG GCA GCG CGT CTC CGC GCG GAG CTG GAG GCG GCC GAG GAG	720
Leu Glu Glu Ala Ala Arg Leu Arg Ala Glu Leu Glu Ala Ala Glu Glu	
225 230 235 240	
GCG GCC CGC CTG GAT GTC ATG CAC GAG GCC GAG CAG GCC CGC GTC CAG	768
Ala Ala Arg Leu Asp Val Met His Glu Ala Glu Gln Ala Arg Val Gln	

81

245	250	255	
GCC CTC GAG GAG GCA GCG CGT CTC CGC GCG GAG CTG GAG GCG GCC GAG Ala Leu Glu Glu Ala Ala Arg Leu Arg Ala Glu Leu Glu Ala Ala Glu 260 265 270			816
GAG GCG GCC CGC CTG GAT GTC ATG CAC GAG GGC GAG CAG GCC CGT GTC Glu Ala Ala Arg Leu Asp Val Met His Glu Gly Glu Gln Ala Arg Val 275 280 285			864
CAG GCC CTC GAG GAG GCG GCC CGC CTG GAG GCC ATG CAC GAG GCC GAG Gln Ala Leu Glu Glu Ala Ala Arg Leu Glu Ala Met His Glu Ala Glu 290 295 300			912
CAG GCC CGC TCC CAG GCC CTC GAG GAG GCA GCG CGT CTC TGC GCG GAG Gln Ala Arg Ser Gln Ala Leu Glu Glu Ala Ala Arg Leu Cys Ala Glu 305 310 315 320			960
CTG GAG GCT GAG GAG GAG GAA AAA GAT GAG CGG CCG GCG ACG TCG AGC Leu Glu Ala Glu Glu Glu Glu Lys Asp Glu Arg Pro Ala Thr Ser Ser 325 330 335			1008
TAC AGC GAG GAG TGC AAA GGG CGA CTG CTA TCG AGG GCG CGG CCG GAT Tyr Ser Glu Glu Cys Lys Gly Arg Leu Leu Ser Arg Ala Arg Pro Asp 340 345 350			1056
CCG CGG AGG CCG CTG CCG CGG CCG TTC ATT GGG ATG TCA CTG TTG GAG Pro Arg Arg Pro Leu Pro Arg Pro Phe Ile Gly Met Ser Leu Leu Glu 355 360 365			1104
GAT GTG GAG AAG AGT ATT CTC ATT GTG GAC GGG CTC TAC AGG GAT GGG Asp Val Glu Lys Ser Ile Leu Ile Val Asp Gly Leu Tyr Arg Asp Gly 370 375 380			1152
CCG GCG TAC CAG ACG GGC ATC CGC CTC GGG GAT GTC CTC TTG CGT ATC Pro Ala Tyr Gln Thr Gly Ile Arg Leu Gly Asp Val Leu Leu Arg Ile 385 390 395 400			1200
GCG GGG GTT TAC GTG GAT TCA ATA GCG AAG GCG AGG CAG GTG GTC GAT Ala Gly Val Tyr Val Asp Ser Ile Ala Lys Ala Arg Gln Val Val Asp 405 410 415			1248
GCG CGT TGC CGC TGC GGC TGC GTC GTT CCC GTG ACG CTG GCG ACG AAG Ala Arg Cys Arg Cys Gly Cys Val Val Pro Val Thr Leu Ala Thr Lys 420 425 430			1296
ATG AAC CAG CAG TAC AGC GTG GCT CTG TAT ATC ATG ACG GTG GAT CCG Met Asn Gln Gln Tyr Ser Val Ala Leu Tyr Ile Met Thr Val Asp Pro 435 440 445			1344
CAG CAC AAC GAC AAG CCC TTT TTT TTT GAT GTG CAC ATC CAC CAC CGC Gln His Asn Asp Lys Pro Phe Phe Phe Asp Val His Ile His His Arg 450 455 460			1392
ATC GAG AGC TCG CAC ATG GGG AAG AAG GCG CAG TGG ATG GAA GTT CTT			1440

82

Ile	Glu	Ser	Ser	His	Met	Gly	Lys	Lys	Ala	Gln	Trp	Met	Glu	Val	Leu	
465					470					475					480	
GAG	AGC	CCA	TCC	GTA	TCT	TCG	GCT	GCC	ACC	ACC	CCT	CTC	GTG	CCG	CTC	1488
Glu	Ser	Pro	Ser	Val	Ser	Ser	Ala	Ala	Thr	Thr	Pro	Leu	Val	Pro	Leu	
				485					490					495		
TTG	CGT	GAG	CCG	ACG	CCG	CGT	AGG	GGC	TCA	GAG	CTG	CAG	TCA	AGT	GCT	1536
Leu	Arg	Glu	Pro	Thr	Pro	Arg	Arg	Gly	Ser	Glu	Leu	Gln	Ser	Ser	Ala	
			500					505					510			
CGT	TCC	GCC	TTC	GTT	GCC	ACG	TCT	TAC	TTC	TCG	AGC	GCG	CGC	AGG	TCG	1584
Arg	Ser	Ala	Phe	Val	Ala	Thr	Ser	Tyr	Phe	Ser	Ser	Ala	Arg	Arg	Ser	
		515					520					525				
GTC	AGC	TCA	GAA	AGT	GAG	CGA	CCG	CGC	GGG	TCC	TCT	AGC	GTG	GCT	ATG	1632
Val	Ser	Ser	Glu	Ser	Glu	Arg	Pro	Arg	Gly	Ser	Ser	Ser	Val	Ala	Met	
	530					535					540					
GCG	GAG	GAG	GCG	ATC	GCG	CTG	GCG	CCG	CAA	GGG	TAT	ACC	CCA	CCC	AAC	1680
Ala	Glu	Glu	Ala	Ile	Ala	Leu	Ala	Pro	Gln	Gly	Tyr	Thr	Pro	Pro	Asn	
545				550					555						560	
CAA	GTG	CGC	GGC	CGT	AGT	TGACGTCTCT	GTGTGAGTGT	GTGTCGCTCC								1728
Gln	Val	Arg	Gly	Arg	Ser											
				565												
GTCTCCTTCC	TTTTTCGTCA	TGTGTTTTAT	TCATTCTTTT	TTC												1771

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 566 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Gln	Ala	Arg	Val	Gln	Ala	Leu	Glu	Glu	Ala	Ala	Arg	Leu	Arg	Ala	Glu
1				5					10					15	
Leu	Glu	Ala	Ala	Glu	Glu	Ala	Ala	Arg	Leu	Asp	Val	Met	His	Ala	Ala
		20						25					30		
Glu	Gln	Ala	Arg	Val	Gln	Ala	Leu	Glu	Glu	Ala	Ala	Arg	Leu	Arg	Ala
		35					40					45			
Glu	Leu	Glu	Glu	Ala	Glu	Glu	Ala	Ala	Arg	Leu	Asp	Val	Met	His	Ala
	50					55				60					
Ala	Glu	Gln	Ala	Arg	Val	Gln	Ala	Leu	Glu	Glu	Ala	Ala	Arg	Leu	Arg
65					70					75				80	

84

Asp Val Glu Lys Ser Ile Leu Ile Val Asp Gly Leu Tyr Arg Asp Gly
 370 375 380
 Pro Ala Tyr Gln Thr Gly Ile Arg Leu Gly Asp Val Leu Leu Arg Ile
 385 390 395 400
 Ala Gly Val Tyr Val Asp Ser Ile Ala Lys Ala Arg Gln Val Val Asp
 405 410 415
 Ala Arg Cys Arg Cys Gly Cys Val Val Pro Val Thr Leu Ala Thr Lys
 420 425 430
 Met Asn Gln Gln Tyr Ser Val Ala Leu Tyr Ile Met Thr Val Asp Pro
 435 440 445
 Gln His Asn Asp Lys Pro Phe Phe Phe Asp Val His Ile His His Arg
 450 455 460
 Ile Glu Ser Ser His Met Gly Lys Lys Ala Gln Trp Met Glu Val Leu
 465 470 475 480
 Glu Ser Pro Ser Val Ser Ser Ala Ala Thr Thr Pro Leu Val Pro Leu
 485 490 495
 Leu Arg Glu Pro Thr Pro Arg Arg Gly Ser Glu Leu Gln Ser Ser Ala
 500 505 510
 Arg Ser Ala Phe Val Ala Thr Ser Tyr Phe Ser Ser Ala Arg Arg Ser
 515 520 525
 Val Ser Ser Glu Ser Glu Arg Pro Arg Gly Ser Ser Ser Val Ala Met
 530 535 540
 Ala Glu Glu Ala Ile Ala Leu Ala Pro Gln Gly Tyr Thr Pro Pro Asn
 545 550 555 560
 Gln Val Arg Gly Arg Ser
 565

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1618 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 115..1323

85

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CCACTCTCTC GGTCTGTCTGT CTCCCACGCG CGCACGCAGT TGATTTCCGC CTTCTTAAAC	60
GCTCTCTTTT TTTTATTTT TCACCTGACC AACCGCACCA CGTCGGCCTC CATC ATG	117
	Met
	1
TCG CAG CAA GAC CGA GTT GCC CCA CAG GAC CAG GAC TCG TTC CTC GAC	165
Ser Gln Gln Asp Arg Val Ala Pro Gln Asp Gln Asp Ser Phe Leu Asp	
5 10 15	
GAC CAG CCC GGC GTC CGC CCG ATC CCG TCC TTC GAT GAC ATG CCG TTG	213
Asp Gln Pro Gly Val Arg Pro Ile Pro Ser Phe Asp Asp Met Pro Leu	
20 25 30	
CAC CAG AAC CTT CTG CGC GGC ATC TAC TCG TAC GGC TTC GAG AAA CCG	261
His Gln Asn Leu Leu Arg Gly Ile Tyr Ser Tyr Gly Phe Glu Lys Pro	
35 40 45	
TCC AGC ATC CAG CAG CGC GCC ATC GCC CCC TTC ACG CGC GGC GGC GAC	309
Ser Ser Ile Gln Gln Arg Ala Ile Ala Pro Phe Thr Arg Gly Gly Asp	
50 55 60 65	
ATC ATC GCG CAG GCG CAG TCC GGT ACC GGC AAG ACG GGC GCC TTC TCC	357
Ile Ile Ala Gln Ala Gln Ser Gly Thr Gly Lys Thr Gly Ala Phe Ser	
70 75 80	
ATC GGC CTG CTG CAG CGC CTG GAC TTC CGC CAC AAC CTG ATC CAG GGC	405
Ile Gly Leu Leu Gln Arg Leu Asp Phe Arg His Asn Leu Ile Gln Gly	
85 90 95	
CTC GTG CTC TCC CCG ACC CGC GAG CTG GCC CTG CAG ACG GCG GAG GTG	453
Leu Val Leu Ser Pro Thr Arg Glu Leu Ala Leu Gln Thr Ala Glu Val	
100 105 110	
ATC AGC CGC ATC GGC GAG TTC CTG TCG AAC AGC GCG AAG TTC TGT GAG	501
Ile Ser Arg Ile Gly Glu Phe Leu Ser Asn Ser Ala Lys Phe Cys Glu	
115 120 125	
ACC TTT GTG GGT GGC ACG CGC GTG CAG GAT GAC CTG CGC AAG CTG CAG	549
Thr Phe Val Gly Gly Thr Arg Val Gln Asp Asp Leu Arg Lys Leu Gln	
130 135 140 145	
GCT GGC GTC GTC GTC GCC GTG GGG ACG CCG GGC CGC GTG TCC GAC GTG	597
Ala Gly Val Val Val Ala Val Gly Thr Pro Gly Arg Val Ser Asp Val	
150 155 160	
ATC AAG CGC GGC GCG CTG CGC ACC GAG TCC CTG CGC GTG CTG GTG CTC	645
Ile Lys Arg Gly Ala Leu Arg Thr Glu Ser Leu Arg Val Leu Val Leu	
165 170 175	
GAC GAG GCT GAT GAG ATG CTG TCT CAG GGC TTC GCG GAT CAG ATT TAC	693
Asp Glu Ala Asp Glu Met Leu Ser Gln Gly Phe Ala Asp Gln Ile Tyr	
180 185 190	

GAG ATC TTC CGC TTC CTG CCG AAG GAC ATC CAG GTC GCG CTC TTC TCC Glu Ile Phe Arg Phe Leu Pro Lys Asp Ile Gln Val Ala Leu Phe Ser 195 200 205	741
GCC ACG ATG CCG GAG GAG GTG CTG GAG CTG ACA AAG AAG TTC ATG CGC Ala Thr Met Pro Glu Glu Val Leu Glu Leu Thr Lys Lys Phe Met Arg 210 215 220 225	789
GAC CCC GTA CGC ATT CTC GTG AAG CGC GAG AGC CTG ACG CTG GAG GGC Asp Pro Val Arg Ile Leu Val Lys Arg Glu Ser Leu Thr Leu Glu Gly 230 235 240	837
ATC AAG CAG TTC TTC ATC GCC GTC GAG GAG GAG CAC AAG CTG GAC ACG Ile Lys Gln Phe Phe Ile Ala Val Glu Glu Glu His Lys Leu Asp Thr 245 250 255	885
CTG ATG GAC CTG TAC GAG ACC GTG TCC ATC GCG CAG TCC GTC ATC TTC Leu Met Asp Leu Tyr Glu Thr Val Ser Ile Ala Gln Ser Val Ile Phe 260 265 270	933
GCC AAC ACC CGC CGC AAG GTG GAC TGG ATC GCC GAG AAG CTG AAT CAG Ala Asn Thr Arg Arg Lys Val Asp Trp Ile Ala Glu Lys Leu Asn Gln 275 280 285	981
AGC AAC CAC ACC GTC AGC AGC ATG CAC GCC GAG ATG CCC AAG AGC GAC Ser Asn His Thr Val Ser Ser Met His Ala Glu Met Pro Lys Ser Asp 290 295 300 305	1029
CGC GAG CGC GTC ATG AAC ACC TTC CGC AGC GGC AGC TCC CGC GTG CTC Arg Glu Arg Val Met Asn Thr Phe Arg Ser Gly Ser Ser Arg Val Leu 310 315 320	1077
GTA ACG ACC GAC CTC GTG GCC CGC GGC ATC GAC GTG CAC CAC GTG AAC Val Thr Thr Asp Leu Val Ala Arg Gly Ile Asp Val His His Val Asn 325 330 335	1125
ATC GTC ATC AAC TTC GAC CTG CCG ACG AAC AAG GAG AAC TAC CTG CAC Ile Val Ile Asn Phe Asp Leu Pro Thr Asn Lys Glu Asn Tyr Leu His 340 345 350	1173
CGC ATT GGC CGC GGC GGC CGC TAC GGC GTA AAG GGT GTT GCC ATC AAC Arg Ile Gly Arg Gly Gly Arg Tyr Gly Val Lys Gly Val Ala Ile Asn 355 360 365	1221
TTC GTG ACG GAG AAA GAC GTG GAG CTG CTG CAC GAG ATC GAG GGG CAC Phe Val Thr Glu Lys Asp Val Glu Leu Leu His Glu Ile Glu Gly His 370 375 380 385	1269
TAC CAC ACG CAG ATC GAT GAG CTC CCG GTG GAC TTT GCC GCC TAC CTC Tyr His Thr Gln Ile Asp Glu Leu Pro Val Asp Phe Ala Ala Tyr Leu 390 395 400	1317
GGC GAG TGA GCGGGCCCCT GCCCCCCTTC CCTGCCCCCC TCTCGCGACG Gly Glu	1366

AGAGAACGCA CATCGTAACA CAGCCACGCG AACGATAGTA AGGGCGTGCG GCGGCGTTCC 1426
 CCTCCTCCTG CCAGCGGGCC CCCTCCGCAG CGCTTCTCTT TTGAGAGGGG GGCAGGGGGA 1486
 GCGCGTGCGC CTGGCTGGAT GTGTGCTTGA GCTTGCAATTC CGTCAAGCAA GTGCTTTGTT 1546
 TTAATTATGC GCGCCGTTTT GTTGCTCGTC CCTTTCGTTG GTGTTTTTTC GGCCGAAACG 1606
 GCGTTTAAAG CA 1618

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 403 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Ser Gln Gln Asp Arg Val Ala Pro Gln Asp Gln Asp Ser Phe Leu
 1 5 10 15
 Asp Asp Gln Pro Gly Val Arg Pro Ile Pro Ser Phe Asp Asp Met Pro
 20 25 30
 Leu His Gln Asn Leu Leu Arg Gly Ile Tyr Ser Tyr Gly Phe Glu Lys
 35 40 45
 Pro Ser Ser Ile Gln Gln Arg Ala Ile Ala Pro Phe Thr Arg Gly Gly
 50 55 60
 Asp Ile Ile Ala Gln Ala Gln Ser Gly Thr Gly Lys Thr Gly Ala Phe
 65 70 75 80
 Ser Ile Gly Leu Leu Gln Arg Leu Asp Phe Arg His Asn Leu Ile Gln
 85 90 95
 Gly Leu Val Leu Ser Pro Thr Arg Glu Leu Ala Leu Gln Thr Ala Glu
 100 105 110
 Val Ile Ser Arg Ile Gly Glu Phe Leu Ser Asn Ser Ala Lys Phe Cys
 115 120 125
 Glu Thr Phe Val Gly Gly Thr Arg Val Gln Asp Asp Leu Arg Lys Leu
 130 135 140
 Gln Ala Gly Val Val Val Ala Val Gly Thr Pro Gly Arg Val Ser Asp
 145 150 155 160
 Val Ile Lys Arg Gly Ala Leu Arg Thr Glu Ser Leu Arg Val Leu Val

88

165 170 175

Leu Asp Glu Ala Asp Glu Met Leu Ser Gln Gly Phe Ala Asp Gln Ile
 180 185 190

Tyr Glu Ile Phe Arg Phe Leu Pro Lys Asp Ile Gln Val Ala Leu Phe
 195 200 205

Ser Ala Thr Met Pro Glu Glu Val Leu Glu Leu Thr Lys Lys Phe Met
 210 215 220

Arg Asp Pro Val Arg Ile Leu Val Lys Arg Glu Ser Leu Thr Leu Glu
 225 230 235 240

Gly Ile Lys Gln Phe Phe Ile Ala Val Glu Glu Glu His Lys Leu Asp
 245 250 255

Thr Leu Met Asp Leu Tyr Glu Thr Val Ser Ile Ala Gln Ser Val Ile
 260 265 270

Phe Ala Asn Thr Arg Arg Lys Val Asp Trp Ile Ala Glu Lys Leu Asn
 275 280 285

Gln Ser Asn His Thr Val Ser Ser Met His Ala Glu Met Pro Lys Ser
 290 295 300

Asp Arg Glu Arg Val Met Asn Thr Phe Arg Ser Gly Ser Ser Arg Val
 305 310 315 320

Leu Val Thr Thr Asp Leu Val Ala Arg Gly Ile Asp Val His His Val
 325 330 335

Asn Ile Val Ile Asn Phe Asp Leu Pro Thr Asn Lys Glu Asn Tyr Leu
 340 345 350

His Arg Ile Gly Arg Gly Gly Arg Tyr Gly Val Lys Gly Val Ala Ile
 355 360 365

Asn Phe Val Thr Glu Lys Asp Val Glu Leu Leu His Glu Ile Glu Gly
 370 375 380

His Tyr His Thr Gln Ile Asp Glu Leu Pro Val Asp Phe Ala Ala Tyr
 385 390 395 400

Leu Gly Glu

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

89

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 6
- (D) OTHER INFORMATION: /note= "Where Xaa is either a Leu or Lys Residue"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Xaa Gln Xaa Pro Gln Xaa Val Phe Asp Glu Xaa Xaa
1 5 10

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: -
- (B) LOCATION: 11
- (D) OTHER INFORMATION: /note= "Where n is inosine"

(ix) FEATURE:

- (A) NAME/KEY: -
- (B) LOCATION: 17
- (D) OTHER INFORMATION: /note= "Where n is inosine"

(ix) FEATURE:

- (A) NAME/KEY: -
- (B) LOCATION: 20
- (D) OTHER INFORMATION: /note= "Where n is inosine"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GGAATTC CCC NCAGCTNGTN TTCGAC

26

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

90

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Lys Val Phe Asp Glu
 1 5

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 30 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GGATCCATGG TCAAGTCCCA CTACATCTGC

30

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 33 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GAATTCAGAC CGGATAGAAA TAAGCCAATG AAA

33

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 701 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Met Thr Glu Thr Phe Ala Phe Gln Ala Glu Ile Asn Gln Leu Met Ser
 1 5 10 15

Leu Ile Ile Asn Thr Phe Tyr Ser Asn Lys Glu Ile Phe Leu Arg Asp

91

20	25	30
Val Ile Ser Asn Ala Ser Asp Ala Cys Asp Lys Ile Arg Tyr Gln Ser		
35	40	45
Leu Thr Asp Pro Ala Val Leu Gly Asp Ala Thr Arg Leu Cys Val Arg		
50	55	60
Val Val Pro Asp Lys Glu Asn Lys Thr Leu Thr Val Glu Asp Asn Gly		
65	70	75 80
Ile Gly Met Thr Lys Ala Asp Leu Val Asn Asn Leu Gly Thr Ile Ala		
	85	90 95
Arg Ser Gly Thr Lys Ala Phe Met Glu Ala Leu Glu Ala Gly Ala Asp		
	100	105 110
Met Ser Met Ile Gly Gln Phe Gly Val Gly Phe Tyr Ser Ala Tyr Leu		
	115	120 125
Val Ala Asp Arg Val Thr Val Thr Ser Lys Asn Asn Ser Asp Glu Val		
	130	135 140
Tyr Val Trp Glu Ser Ser Ala Gly Gly Thr Phe Thr Ile Thr Ser Ala		
	145	150 155 160
Pro Glu Ser Asp Met Lys Leu Pro Ala Arg Ile Thr Leu His Leu Lys		
	165	170 175
Glu Asp Gln Leu Glu Tyr Leu Glu Ala Arg Arg Leu Lys Glu Leu Ile		
	180	185 190
Lys Lys His Ser Glu Phe Ile Gly Tyr Asp Ile Glu Leu Met Val Glu		
	195	200 205
Lys Thr Thr Glu Lys Glu Val Thr Asp Glu Asp Glu Glu Glu Ala Lys		
	210	215 220
Lys Ala Asp Glu Asp Gly Glu Glu Pro Lys Val Glu Glu Val Thr Glu		
	225	230 235 240
Gly Glu Glu Asp Lys Lys Lys Lys Thr Lys Lys Val Lys Glu Val Thr		
	245	250 255
Lys Glu Tyr Glu Val Gln Asn Lys His Lys Pro Leu Trp Thr Arg Asp		
	260	265 270
Pro Lys Asp Val Thr Lys Glu Glu Tyr Ala Ala Phe Tyr Lys Ala Ile		
	275	280 285
Ser Asn Asp Trp Glu Asp Pro Pro Ala Thr Lys His Phe Ser Val Glu		
	290	295 300
Gly Gln Leu Glu Phe Arg Ala Ile Met Phe Val Pro Lys Arg Ala Pro		
	305	310 315 320

Phe Asp Met Leu Glu Pro Asn Lys Lys Arg Asn Asn Ile Lys Leu Tyr
 325 330 335
 Val Arg Arg Val Phe Ile Met Asp Asn Cys Glu Asp Leu Cys Pro Asp
 340 345 350
 Trp Leu Gly Phe Val Lys Gly Val Val Asp Ser Glu Asp Leu Pro Leu
 355 360 365
 Asn Ile Ser Arg Glu Asn Leu Gln Gln Asn Lys Ile Leu Lys Val Ile
 370 375 380
 Arg Lys Asn Ile Val Lys Lys Cys Leu Glu Met Phe Glu Glu Val Ala
 385 390 395 400
 Glu Asn Lys Glu Asp Tyr Lys Gln Phe Tyr Glu Gln Phe Gly Lys Asn
 405 410 415
 Ile Lys Leu Gly Ile His Glu Asp Thr Ala Asn Arg Lys Lys Leu Met
 420 425 430
 Glu Leu Leu Arg Phe Tyr Ser Thr Glu Ser Gly Glu Val Met Thr Thr
 435 440 445
 Leu Lys Asp Tyr Val Thr Arg Met Lys Ala Glu Gln Asn Ser Ile Tyr
 450 455 460
 Tyr Ile Thr Gly Asp Ser Lys Lys Lys Leu Glu Ser Ser Pro Phe Ile
 465 470 475 480
 Glu Gln Ala Lys Arg Arg Gly Phe Glu Val Leu Phe Met Thr Glu Pro
 485 490 495
 Tyr Asp Glu Tyr Val Met Gln Gln Val Lys Asp Phe Glu Asp Lys Lys
 500 505 510
 Phe Ala Cys Leu Thr Lys Glu Gly Val His Phe Glu Glu Ser Glu Glu
 515 520 525
 Glu Lys Lys Gln Arg Glu Glu Glu Lys Ala Thr Cys Glu Lys Leu Cys
 530 535 540
 Lys Thr Met Lys Glu Val Leu Gly Asp Lys Val Glu Lys Val Thr Val
 545 550 555 560
 Ser Glu Arg Leu Ser Thr Ser Pro Cys Ile Leu Val Thr Ser Glu Phe
 565 570 575
 Gly Trp Ser Ala His Met Glu Gln Met Met Arg Asn Gln Ala Leu Arg
 580 585 590
 Asp Ser Ser Met Ala Gln Tyr Met Met Ser Lys Lys Thr Met Glu Leu
 595 600 605

93

Asn Pro Lys His Pro Ile Ile Lys Glu Leu Arg Arg Arg Val Glu Ala
 610 615 620
 Asp Glu Asn Asp Lys Ala Val Lys Asp Leu Val Phe Leu Leu Phe Asp
 625 630 635 640
 Thr Ser Leu Leu Thr Ser Gly Phe Gln Leu Glu Asp Pro Thr Tyr Ala
 645 650 655
 Glu Arg Ile Asn Arg Met Ile Lys Leu Gly Leu Ser Leu Asp Glu Glu
 660 665 670
 Glu Glu Glu Glu Ala Val Glu Ala Ala Val Ala Glu Thr Ala Pro Ala
 675 680 685
 Glu Val Thr Ala Gly Thr Ser Ser Met Glu Leu Val Asp
 690 695 700

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 704 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Met Thr Glu Thr Phe Ala Phe Gln Ala Glu Ile Asn Gln Leu Met Ser
 1 5 10 15
 Leu Ile Ile Asn Thr Phe Tyr Ser Asn Lys Glu Ile Phe Leu Arg Glu
 20 25 30
 Leu Ile Ser Asn Ala Ser Asp Ala Cys Asp Lys Ile Arg Tyr Gln Ser
 35 40 45
 Leu Thr Asn Gln Ala Val Leu Gly Asp Glu Ser His Leu Arg Ile Arg
 50 55 60
 Val Val Pro Asp Lys Ala Asn Lys Thr Leu Thr Val Glu Asp Thr Gly
 65 70 75 80
 Ile Gly Met Thr Lys Ala Glu Leu Val Asn Asn Leu Gly Thr Ile Ala
 85 90 95
 Arg Ser Gly Thr Lys Ala Phe Met Glu Ala Leu Glu Ala Gly Gly Asp
 100 105 110
 Met Ser Met Ile Gly Gln Phe Gly Val Gly Phe Tyr Ser Ala Tyr Leu
 115 120 125

94

Val Ala Asp Arg Val Thr Val Val Ser Lys Asn Asn Asp Asp Glu Ala
 130 135 140

Tyr Thr Trp Glu Ser Ser Ala Gly Gly Thr Phe Thr Val Thr Pro Thr
 145 150 155 160

Pro Asp Cys Asp Leu Lys Arg Gly Thr Arg Ile Val Leu His Leu Lys
 165 170 175

Glu Asp Gln Gln Glu Tyr Leu Glu Glu Arg Arg Leu Lys Asp Leu Ile
 180 185 190

Lys Lys His Ser Glu Phe Ile Gly Tyr Asp Ile Glu Leu Met Val Glu
 195 200 205

Lys Ala Thr Glu Lys Glu Val Thr Asp Glu Asp Glu Asp Glu Ala Ala
 210 215 220

Ala Thr Lys Asn Glu Glu Gly Glu Glu Pro Lys Val Glu Glu Val Lys
 225 230 235 240

Asp Asp Ala Glu Glu Gly Glu Lys Lys Lys Lys Thr Lys Lys Val Lys
 245 250 255

Glu Val Thr Gln Glu Phe Val Val Gln Asn Lys His Lys Pro Leu Trp
 260 265 270

Thr Arg Asp Pro Lys Asp Val Thr Lys Glu Glu Tyr Ala Ala Phe Tyr
 275 280 285

Lys Ala Ile Ser Asn Asp Trp Glu Glu Pro Leu Ser Thr Lys His Phe
 290 295 300

Ser Val Glu Gly Gln Leu Glu Phe Arg Ala Ile Leu Phe Val Pro Lys
 305 310 315 320

Arg Ala Pro Phe Asp Met Phe Glu Pro Ser Lys Lys Arg Asn Asn Ile
 325 330 335

Lys Leu Tyr Val Arg Arg Val Phe Ile Met Asp Asn Cys Glu Asp Leu
 340 345 350

Cys Pro Glu Trp Leu Ala Phe Val Arg Gly Val Val Asp Ser Glu Asp
 355 360 365

Leu Pro Leu Asn Ile Ser Arg Glu Asn Leu Gln Gln Asn Lys Ile Leu
 370 375 380

Lys Val Ile Arg Lys Asn Ile Val Lys Lys Ala Leu Glu Leu Phe Glu
 385 390 395 400

Glu Ile Ala Glu Asn Lys Glu Asp Tyr Lys Lys Phe Tyr Glu Gln Phe
 405 410 415

95

Gly	Lys	Asn	Val	Lys	Leu	Gly	Ile	His	Glu	Asp	Ser	Ala	Asn	Arg	Lys	420	425	430
Lys	Leu	Met	Glu	Leu	Leu	Arg	Phe	His	Ser	Ser	Glu	Ser	Gly	Glu	Asp	435	440	445
Met	Thr	Thr	Leu	Lys	Asp	Tyr	Val	Thr	Arg	Met	Lys	Glu	Gly	Gln	Lys	450	455	460
Cys	Ile	Tyr	Tyr	Val	Thr	Gly	Asp	Ser	Lys	Lys	Lys	Leu	Glu	Thr	Ser	465	470	475
Pro	Phe	Ile	Glu	Gln	Ala	Arg	Arg	Arg	Gly	Phe	Glu	Val	Leu	Phe	Met	485	490	495
Thr	Glu	Pro	Ile	Asp	Glu	Tyr	Val	Met	Gln	Gln	Val	Lys	Asp	Phe	Glu	500	505	510
Asp	Lys	Lys	Phe	Ala	Cys	Leu	Thr	Lys	Glu	Gly	Val	His	Phe	Glu	Glu	515	520	525
Thr	Glu	Glu	Glu	Lys	Lys	Gln	Arg	Glu	Glu	Glu	Lys	Thr	Ala	Tyr	Glu	530	535	540
Arg	Leu	Cys	Lys	Ala	Met	Lys	Asp	Val	Leu	Gly	Asp	Lys	Val	Glu	Lys	545	550	555
Val	Val	Val	Ser	Glu	Arg	Leu	Ala	Thr	Ser	Pro	Cys	Ile	Leu	Val	Thr	565	570	575
Ser	Glu	Phe	Gly	Trp	Ser	Ala	His	Met	Glu	Gln	Ile	Met	Arg	Asn	Gln	580	585	590
Ala	Leu	Arg	Asp	Ser	Ser	Met	Ser	Ala	Tyr	Met	Met	Ser	Lys	Lys	Thr	595	600	605
Met	Glu	Ile	Asn	Pro	Ala	His	Pro	Ile	Val	Lys	Glu	Leu	Lys	Arg	Arg	610	615	620
Val	Glu	Ala	Asp	Glu	Asn	Asp	Lys	Ala	Val	Lys	Asp	Leu	Val	Tyr	Leu	625	630	635
Leu	Phe	Asp	Thr	Ala	Leu	Leu	Thr	Ser	Gly	Phe	Thr	Leu	Asp	Asp	Pro	645	650	655
Thr	Ser	Tyr	Ala	Glu	Arg	Ile	His	Arg	Met	Ile	Lys	Leu	Gly	Leu	Ser	660	665	670
Leu	Asp	Asp	Glu	Asp	Asn	Gly	Asn	Glu	Glu	Ala	Glu	Pro	Ala	Ala	Ala	675	680	685
Val	Pro	Ala	Glu	Pro	Val	Ala	Gly	Thr	Ser	Ser	Met	Glu	Gln	Val	Asp	690	695	700

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 732 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

```

Met Pro Glu Glu Thr Gln Thr Gln Asp Gln Pro Met Glu Glu Glu Glu
1           5           10           15

Val Glu Thr Phe Ala Phe Gln Ala Glu Ile Ala Gln Leu Met Ser Leu
20           25           30

Ile Ile Asn Thr Phe Tyr Ser Asn Lys Glu Ile Phe Leu Arg Glu Leu
35           40           45

Ile Ser Asn Ser Ser Asp Ala Leu Asp Lys Ile Arg Tyr Glu Ser Leu
50           55           60

Thr Asp Pro Ser Lys Leu Asp Ser Gly Lys Glu Leu His Ile Asn Leu
65           70           75           80

Ile Pro Asn Lys Gln Asp Arg Ala Leu Thr Ile Val Asp Thr Gly Ile
85           90           95

Gly Met Thr Lys Ala Asp Leu Ile Asn Asn Leu Gly Thr Ile Ala Lys
100          105          110

Ser Gly Thr Lys Ala Phe Met Glu Ala Leu Gln Ala Gly Ala Asp Ile
115          120          125

Ser Met Ile Gly Gln Phe Gly Val Gly Phe Tyr Ser Ala Tyr Leu Val
130          135          140

Ala Glu Lys Val Thr Val Ile Thr Lys His Asn Asp Asp Glu Gln Tyr
145          150          155          160

Ala Trp Glu Ser Ser Ala Gly Gly Ser Phe Thr Val Arg Thr Asp Thr
165          170          175

Gly Glu Pro Met Gly Arg Gly Thr Lys Val Ile Leu His Leu Lys Glu
180          185          190

Asp Gln Thr Glu Tyr Leu Glu Glu Arg Arg Ile Lys Glu Ile Val Lys
195          200          205

Lys His Ser Gln Phe Ile Gly Tyr Pro Ile Thr Leu Phe Val Glu Lys
210          215          220

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Glu Arg Asp Lys Glu Val Ser Asp Asp Glu Ala Glu Glu Lys Glu Asp
 225 230 235 240
 Lys Glu Glu Glu Lys Glu Lys Glu Glu Lys Glu Ser Glu Asp Lys Pro
 245 250 255
 Glu Ile Glu Asp Val Gly Ser Asp Glu Glu Asp Glu Lys Lys Asp Gly
 260 265 270
 Asp Lys Lys Lys Lys Lys Lys Ile Lys Glu Lys Tyr Ile Asp Lys Glu
 275 280 285
 Glu Leu Asn Lys Thr Lys Pro Ile Trp Thr Arg Asn Pro Asp Asp Ile
 290 295 300
 Thr Asn Glu Glu Tyr Gly Glu Phe Tyr Lys Ser Leu Thr Asn Asp Trp
 305 310 315 320
 Glu Asp His Leu Ala Val Lys His Phe Ser Val Glu Gly Gln Leu Glu
 325 330 335
 Phe Arg Ala Leu Leu Phe Val Pro Arg Arg Ala Pro Phe Asp Leu Phe
 340 345 350
 Glu Asn Arg Lys Lys Lys Asn Asn Ile Lys Leu Tyr Val Arg Arg Val
 355 360 365
 Phe Ile Met Asp Asn Cys Glu Glu Leu Ile Pro Glu Tyr Leu Asn Phe
 370 375 380
 Ile Arg Gly Val Val Asp Ser Glu Asp Leu Pro Leu Asn Ile Ser Arg
 385 390 395 400
 Glu Met Leu Gln Gln Ser Lys Ile Leu Lys Val Ile Arg Lys Asn Leu
 405 410 415
 Val Lys Lys Cys Leu Glu Leu Phe Thr Glu Leu Ala Glu Asp Lys Glu
 420 425 430
 Asn Tyr Lys Lys Phe Tyr Glu Gln Phe Ser Lys Asn Ile Lys Leu Gly
 435 440 445
 Ile His Glu Asp Ser Gln Asn Arg Lys Lys Leu Ser Glu Leu Leu Arg
 450 455 460
 Tyr Tyr Thr Ser Ala Ser Gly Asp Glu Met Val Ser Leu Lys Asp Tyr
 465 470 475 480
 Cys Thr Arg Met Lys Glu Asn Gln Lys His Ile Tyr Tyr Ile Thr Gly
 485 490 495
 Glu Thr Lys Asp Gln Val Ala Asn Ser Ala Phe Val Glu Arg Leu Arg
 500 505 510

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Lys His Gly Leu Glu Val Ile Tyr Met Ile Glu Pro Ile Asp Glu Tyr
 515 520 525
 Cys Val Gln Gln Leu Lys Glu Phe Glu Gly Lys Thr Leu Val Ser Val
 530 535 540
 Thr Lys Glu Gly Leu Glu Leu Pro Glu Asp Glu Glu Glu Lys Lys Lys
 545 550 555 560
 Gln Glu Glu Lys Lys Thr Lys Phe Glu Asn Leu Cys Lys Ile Met Lys
 565 570 575
 Asp Ile Leu Glu Lys Lys Val Glu Lys Val Val Val Ser Asn Arg Leu
 580 585 590
 Val Thr Ser Pro Cys Cys Leu Val Thr Ser Thr Tyr Gly Trp Thr Ala
 595 600 605
 Asn Met Glu Arg Ile Met Lys Ala Gln Ala Leu Arg Asp Asn Ser Thr
 610 615 620
 Met Gly Tyr Met Ala Ala Lys Lys His Leu Glu Ile Asn Pro Asp His
 625 630 635 640
 Ser Ile Ile Glu Thr Leu Arg Gln Lys Ala Glu Ala Asp Lys Asn Asp
 645 650 655
 Lys Ser Val Lys Asp Leu Val Ile Leu Leu Tyr Glu Thr Ala Leu Leu
 660 665 670
 Ser Ser Gly Phe Ser Leu Glu Asp Pro Gln Thr His Ala Asn Arg Ile
 675 680 685
 Tyr Arg Met Ile Lys Leu Gly Leu Gly Ile Asp Glu Asp Asp Pro Thr
 690 695 700
 Ala Asp Asp Thr Ser Ala Ala Val Thr Glu Glu Met Pro Pro Leu Glu
 705 710 715 720
 Gly Asp Asp Asp Thr Ser Arg Met Glu Glu Val Asp
 725 730

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1019 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Leishmania major*

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(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 71..523

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GAATTCGGCA CGAGGTTTCT GTACTTTATT GCTTCCAGCC TTTATTCACT CTTCGATTTC	60
CTCTAACACC ATG TCC TCC GAG CGC ACC TTT ATT GCC GTC AAG CCG GAC	109
Met Ser Ser Glu Arg Thr Phe Ile Ala Val Lys Pro Asp	
1 5 10	
GCC GTG CAG CGC GGC CTC GTT GGC GAG ATC ATC GCC CGC TTC GAG CGC	157
Gly Val Gln Arg Gly Leu Val Gly Glu Ile Ile Ala Arg Phe Glu Arg	
15 20 25	
AAG GGC TAC AAG CTC GTC GCC TTG AAG ATA CTG CAG CCG ACG ACG GAG	205
Lys Gly Tyr Lys Leu Val Ala Leu Lys Ile Leu Gln Pro Thr Thr Glu	
30 35 40 45	
CAG GCC CAG GGT CAC TAT AAG GAC CTT TGC TCC AAG CCG TTT TTC CCG	253
Gln Ala Gln Gly His Tyr Lys Asp Leu Cys Ser Lys Pro Phe Phe Pro	
50 55 60	
GCC CTT GTG AAG TAC TTC TCC TCT GGC CCG ATC GTG TGT ATG GTG TGG	301
Ala Leu Val Lys Tyr Phe Ser Ser Gly Pro Ile Val Cys Met Val Trp	
65 70 75	
GAG GGT AAG AAC GTG GTG AAG AGC GGC CGC GTG CTG CTC GGC GCG ACG	349
Glu Gly Lys Asn Val Val Lys Ser Gly Arg Val Leu Leu Gly Ala Thr	
80 85 90	
AAC CCG GCC GAC TCA CAG CCC GGC ACG ATC CGT GGC GAC TTT GCC GTG	397
Asn Pro Ala Asp Ser Gln Pro Gly Thr Ile Arg Gly Asp Phe Ala Val	
95 100 105	
GAT GTG GGC CGC AAC GTG TGC CAC GGG TCC GAC TCT GTG GAG AGC GCG	445
Asp Val Gly Arg Asn Val Cys His Gly Ser Asp Ser Val Glu Ser Ala	
110 115 120 125	
GAG CGC GAG ATC GCC TTT TGG TTC AAG GCG GAT GAG ATC GCG AGC TGG	493
Glu Arg Glu Ile Ala Phe Trp Phe Lys Ala Asp Glu Ile Ala Ser Trp	
130 135 140	
ACG TCG CAC TCC GTG TCC CAG ATC TAT GAG TAACGGTGAT TGC GGACACG	543
Thr Ser His Ser Val Ser Gln Ile Tyr Glu	
145 150	
CTTTGAGGAC GTAGCTGTAC CCCC AATGAA TTCTTCTCTG AAAACCACAT CATAAGCCTC	603
TTAAGAGGTT ATTTTCTTG ATCGATGCCC GGTGGTGACC AGCACCATTCTTTATCGGA	663
TTCACTCACA CTCCTAGCGA ATCATGTAGT GCGGTGAGAG TGGGCTCTGG AGGAGACTGT	723

100

TGTGTAGCCA TGGCTTCAGG AGAGAAAACA AAATACAAGG AAAGGCAATA TGTAACATATG 783
 GGGTTCCCTT TTTTACTATG CAAAGTTTTT ATAACCTCTG ATCGGCAAAA ACAACAACAA 843
 CCGCCATACA CCAAGAGCAA ATGCTTTCTT CTGCGGACTG TGCTTCTGTT TTTTTTTATG 903
 AAGGAGTGAC TCGCGCGATG AAAAGTGTGT GCGTGGGAGA TGTATTTCTT TTTTTTGTTT 963
 ATAGTGGCGA CAGCTCACTG TTGACGATGA CAAAAAAAAA AAAAAAAAAA CTCGAG 1019

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 151 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Met Ser Ser Glu Arg Thr Phe Ile Ala Val Lys Pro Asp Gly Val Gln
 1 5 10 15
 Arg Gly Leu Val Gly Glu Ile Ile Ala Arg Phe Glu Arg Lys Gly Tyr
 20 25 30
 Lys Leu Val Ala Leu Lys Ile Leu Gln Pro Thr Thr Glu Gln Ala Gln
 35 40 45
 Gly His Tyr Lys Asp Leu Cys Ser Lys Pro Phe Phe Pro Ala Leu Val
 50 55 60
 Lys Tyr Phe Ser Ser Gly Pro Ile Val Cys Met Val Trp Glu Gly Lys
 65 70 75 80
 Asn Val Val Lys Ser Gly Arg Val Leu Leu Gly Ala Thr Asn Pro Ala
 85 90 95
 Asp Ser Gln Pro Gly Thr Ile Arg Gly Asp Phe Ala Val Asp Val Gly
 100 105 110
 Arg Asn Val Cys His Gly Ser Asp Ser Val Glu Ser Ala Glu Arg Glu
 115 120 125
 Ile Ala Phe Trp Phe Lys Ala Asp Glu Ile Ala Ser Trp Thr Ser His
 130 135 140
 Ser Val Ser Gln Ile Tyr Glu
 145 150

(2) INFORMATION FOR SEQ ID NO:21:

101

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1523 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Leishmania major*

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 14..973

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GAATTCGGCA CGA GTG CTG CCC GAC ATG ACA TGC TCG CTG ACC GGA CTT	49
Val Leu Pro Asp Met Thr Cys Ser Leu Thr Gly Leu	
1 5 10	
CAG TGC ACA GAC CCG AAC TGC AAG ACC TGC ACA ACT TAC GGT CAG TGC	97
Gln Cys Thr Asp Pro Asn Cys Lys Thr Cys Thr Thr Tyr Gly Gln Cys	
15 20 25	
ACA GAC TGC AAC GAC GGC TAC GGT CTC ACC TCC TCC AGC GTT TGC GTG	145
Thr Asp Cys Asn Asp Gly Tyr Gly Leu Thr Ser Ser Ser Val Cys Val	
30 35 40	
CGC TGC AGT GTA GCG GGC TGC AAG AGC TGC CCC GTC GAC GCT AAC GTC	193
Arg Cys Ser Val Ala Gly Cys Lys Ser Cys Pro Val Asp Ala Asn Val	
45 50 55 60	
TGC AAA GTG TGT CTC GGC GGC AGC GAG CCG ATC AAC AAT ATG TGC CCC	241
Cys Lys Val Cys Leu Gly Gly Ser Glu Pro Ile Asn Asn Met Cys Pro	
65 70 75	
TGC ACC GAC CCC AAC TGC GCC AGC TGC CCC AGC GAC GCT GGC ACG TGC	289
Cys Thr Asp Pro Asn Cys Ala Ser Cys Pro Ser Asp Ala Gly Thr Cys	
80 85 90	
ACT CAG TGC GCG AAC GGC TAC GGT CTC GTG GAC GGC GCC TGT GTG AGA	337
Thr Gln Cys Ala Asn Gly Tyr Gly Leu Val Asp Gly Ala Cys Val Arg	
95 100 105	
TGC CAG GAG CCC AAC TGC TTC AGC TGC GAC AGC GAC GCG AAT AAG TGC	385
Cys Gln Glu Pro Asn Cys Phe Ser Cys Asp Ser Asp Ala Asn Lys Cys	
110 115 120	
ACA CAA TGT GCG CCG AAC TAC TAC CTC ACC CCG CTC TTG ACC TGC TCC	433
Thr Gln Cys Ala Pro Asn Tyr Tyr Leu Thr Pro Leu Leu Thr Cys Ser	
125 130 135 140	
CCG GTG GCC TGC AAC ATC GAG CAC TGC ATG CAG TGC GAC CCA CAG ACG	481

Pro	Val	Ala	Cys	Asn	Ile	Glu	His	Cys	Met	Gln	Cys	Asp	Pro	Gln	Thr		
				145					150						155		
CCG	TCG	CGC	TGC	CAG	GAG	TGC	GTG	TCC	CCC	TAC	GTG	GTT	GAC	AGC	TAC	529	
Pro	Ser	Arg	Cys	Gln	Glu	Cys	Val	Ser	Pro	Tyr	Val	Val	Asp	Ser	Tyr		
			160					165					170				
GAC	GGC	CTC	TGC	AGG	CTC	TCC	GAT	GCC	TGC	TCC	GTG	CCC	AAC	TGC	AAG	577	
Asp	Gly	Leu	Cys	Arg	Leu	Ser	Asp	Ala	Cys	Ser	Val	Pro	Asn	Cys	Lys		
		175					180					185					
AAG	TGC	GAG	ACC	GGT	ACC	TCC	AGG	CTC	TGC	GCC	GAG	TGC	GAC	ACC	GGC	625	
Lys	Cys	Glu	Thr	Gly	Thr	Ser	Arg	Leu	Cys	Ala	Glu	Cys	Asp	Thr	Gly		
	190					195					200						
TAC	AGT	CTC	TCC	GCC	GAC	GCG	ACG	AGC	TGC	AGC	AGT	CCA	ACC	ACG	CAG	673	
Tyr	Ser	Leu	Ser	Ala	Asp	Ala	Thr	Ser	Cys	Ser	Ser	Pro	Thr	Thr	Gln		
205				210					215						220		
CCG	TGC	GAG	GTG	GAG	CAC	TGC	AAC	ACA	TGT	GTG	AAC	GGC	GAT	AGC	ACC	721	
Pro	Cys	Glu	Val	Glu	His	Cys	Asn	Thr	Cys	Val	Asn	Gly	Asp	Ser	Thr		
			225					230					235				
CGC	TGT	GCC	TAC	TGC	AAC	ACC	GGC	TAC	TAC	GTC	TCC	GAT	GGC	AAG	TGC	769	
Arg	Cys	Ala	Tyr	Cys	Asn	Thr	Gly	Tyr	Tyr	Val	Ser	Asp	Gly	Lys	Cys		
		240					245					250					
AAG	GCC	ATG	CAG	GGC	TGC	TAC	GTG	TCG	AAC	TGC	GCG	CAG	TGC	ATG	CTG	817	
Lys	Ala	Met	Gln	Gly	Cys	Tyr	Val	Ser	Asn	Cys	Ala	Gln	Cys	Met	Leu		
		255				260						265					
CTT	GAC	AGC	ACC	AAG	TGC	TCC	ACG	TGC	GTG	AAA	GGG	TAC	CTG	CTC	ACG	865	
Leu	Asp	Ser	Thr	Lys	Cys	Ser	Thr	Cys	Val	Lys	Gly	Tyr	Leu	Leu	Thr		
	270					275					280						
TCG	TCC	TAC	AGT	TGC	GTC	TCG	CAG	AAA	GTC	ATC	AAC	AGT	GCG	GCC	GCG	913	
Ser	Ser	Tyr	Ser	Cys	Val	Ser	Gln	Lys	Val	Ile	Asn	Ser	Ala	Ala	Ala		
285				290				295						300			
CCC	TAC	TCT	CTG	TGG	GTG	GCC	GCC	GCC	GTG	CTC	CTC	ACC	TCT	TTT	GCC	961	
Pro	Tyr	Ser	Leu	Trp	Val	Ala	Ala	Ala	Val	Leu	Leu	Thr	Ser	Phe	Ala		
			305					310					315				
ATG	CAC	CTA	GCA	TAGTGC	CGCAG	CGGCATG	CGA	ACAACCC	CAC	TCTCAT	TCTC					1013	
Met	His	Leu	Ala														
			320														
CAACATGTGC	ATACACACAC	ACACAGACAG	CGGGGCAGCA	CCCCCTCCCC	ACACACACAC											1073	
ACGCACTTCC	CCCTTGCTTT	GTTCTTCTTT	CCTCGTTTCGC	ATTTCTTTCT	CTCGTGCGCT											1133	
GGCGCCGGCC	TCCTGCACGT	CGCTCCCCCTC	CCCCTAACCT														

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CCACAGTCCC ACAGCGCAGA CACACGTGTT TAAACGGCGC AGGCATCCCT CCCTATCACT 1313
 TCATTTCTCC TAAAGCCACT CACCAAGTCG CACACCGCCC TCCCCCATCG GCCGCCCTTC 1373
 CGGGCGCAGC TGTGCGGAAT GGGTGTGTGC TCGACCTCGT TCCTGGCAGC TCACTCGCAT 1433
 GTGTACAGCC ACTCCAACCA CGAAAGCTCT CTTCTGCGCA CATAAAAAAA AAAAAAAAAA 1493
 AAAAACTCGA GGGGGGGCCC GGTACCCAAA 1523

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 320 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Val Leu Pro Asp Met Thr Cys Ser Leu Thr Gly Leu Gln Cys Thr Asp
 1 5 10 15
 Pro Asn Cys Lys Thr Cys Thr Thr Tyr Gly Gln Cys Thr Asp Cys Asn
 20 25 30
 Asp Gly Tyr Gly Leu Thr Ser Ser Ser Val Cys Val Arg Cys Ser Val
 35 40 45
 Ala Gly Cys Lys Ser Cys Pro Val Asp Ala Asn Val Cys Lys Val Cys
 50 55 60
 Leu Gly Gly Ser Glu Pro Ile Asn Asn Met Cys Pro Cys Thr Asp Pro
 65 70 75 80
 Asn Cys Ala Ser Cys Pro Ser Asp Ala Gly Thr Cys Thr Gln Cys Ala
 85 90 95
 Asn Gly Tyr Gly Leu Val Asp Gly Ala Cys Val Arg Cys Gln Glu Pro
 100 105 110
 Asn Cys Phe Ser Cys Asp Ser Asp Ala Asn Lys Cys Thr Gln Cys Ala
 115 120 125
 Pro Asn Tyr Tyr Leu Thr Pro Leu Leu Thr Cys Ser Pro Val Ala Cys
 130 135 140
 Asn Ile Glu His Cys Met Gln Cys Asp Pro Gln Thr Pro Ser Arg Cys
 145 150 155 160
 Gln Glu Cys Val Ser Pro Tyr Val Val Asp Ser Tyr Asp Gly Leu Cys
 165 170 175

104

Arg	Leu	Ser	Asp	Ala	Cys	Ser	Val	Pro	Asn	Cys	Lys	Lys	Cys	Glu	Thr
			180					185					190		
Gly	Thr	Ser	Arg	Leu	Cys	Ala	Glu	Cys	Asp	Thr	Gly	Tyr	Ser	Leu	Ser
		195					200					205			
Ala	Asp	Ala	Thr	Ser	Cys	Ser	Ser	Pro	Thr	Thr	Gln	Pro	Cys	Glu	Val
	210					215					220				
Glu	His	Cys	Asn	Thr	Cys	Val	Asn	Gly	Asp	Ser	Thr	Arg	Cys	Ala	Tyr
225					230				235						240
Cys	Asn	Thr	Gly	Tyr	Tyr	Val	Ser	Asp	Gly	Lys	Cys	Lys	Ala	Met	Gln
			245						250					255	
Gly	Cys	Tyr	Val	Ser	Asn	Cys	Ala	Gln	Cys	Met	Leu	Leu	Asp	Ser	Thr
		260						265					270		
Lys	Cys	Ser	Thr	Cys	Val	Lys	Gly	Tyr	Leu	Leu	Thr	Ser	Ser	Tyr	Ser
		275					280					285			
Cys	Val	Ser	Gln	Lys	Val	Ile	Asn	Ser	Ala	Ala	Ala	Pro	Tyr	Ser	Leu
	290					295					300				
Trp	Val	Ala	Ala	Ala	Val	Leu	Leu	Thr	Ser	Phe	Ala	Met	His	Leu	Ala
305					310					315					320

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 797 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Leishmania major

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 27..623

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

CTGTACTTTA	TTGCCACCAG	CCAGCC	ATG	TCC	TGC	GGT	AAC	GCC	AAG	ATC	AAC	53				
			Met	Ser	Cys	Gly	Asn	Ala	Lys	Ile	Asn					
			1				5									
TCT	CCC	GCG	CCG	TCC	TTC	GAG	GAG	GTG	GCG	CTC	ATG	CCC	AAC	GGC	AGC	101
Ser	Pro	Ala	Pro	Ser	Phe	Glu	Glu	Val	Ala	Leu	Met	Pro	Asn	Gly	Ser	
10					15				20					25		

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TTC AAG AAG ATC AGC CTC TCC TCC TAC AAG GGC AAG TGG GTC GTG CTC	149
Phe Lys Lys Ile Ser Leu Ser Ser Tyr Lys Gly Lys Trp Val Val Leu	
30 35 40	
TTC TTC TAC CCG CTC GAC TTT AGC TTC GTG TGC CCG ACA GAG GTC ATC	197
Phe Phe Tyr Pro Leu Asp Phe Ser Phe Val Cys Pro Thr Glu Val Ile	
45 50 55	
GCG TTC TCC GAC AGC GTG AGT CGC TTC AAC GAG CTC AAC TGC GAG GTC	245
Ala Phe Ser Asp Ser Val Ser Arg Phe Asn Glu Leu Asn Cys Glu Val	
60 65 70	
CTC GCG TGC TCG ATA GAC AGC GAG TAC GCG CAC CTG CAG TGG ACG CTG	293
Leu Ala Cys Ser Ile Asp Ser Glu Tyr Ala His Leu Gln Trp Thr Leu	
75 80 85	
CAG GAC CGC AAG AAG GGC GGC CTC GGG ACC ATG GCG ATC CCA ATG CTA	341
Gln Asp Arg Lys Lys Gly Gly Leu Gly Thr Met Ala Ile Pro Met Leu	
90 95 100 105	
GCC GAC AAG ACC AAG AGC ATC GCT CGT TCC TAC GGC GTG CTG GAG GAG	389
Ala Asp Lys Thr Lys Ser Ile Ala Arg Ser Tyr Gly Val Leu Glu Glu	
110 115 120	
AGC CAG GGC GTG GCC TAC CGC GGT CTC TTC ATC ATC GAC CCC CAT GGC	437
Ser Gln Gly Val Ala Tyr Arg Gly Leu Phe Ile Ile Asp Pro His Gly	
125 130 135	
ATG CTG CGT CAG ATC ACC GTC AAT GAC ATG CCG GTG GGC CGC AGC GTG	485
Met Leu Arg Gln Ile Thr Val Asn Asp Met Pro Val Gly Arg Ser Val	
140 145 150	
GAG GAG GTT CTA CGC CTG CTG GAG GCT TTT CAG TTC GTG GAG AAG CAC	533
Glu Glu Val Leu Arg Leu Leu Glu Ala Phe Gln Phe Val Glu Lys His	
155 160 165	
GGC GAG GTG TGC CCC GCG AAC TGG AAG AAG GGC GCC CCC ACG ATG AAG	581
Gly Glu Val Cys Pro Ala Asn Trp Lys Lys Gly Ala Pro Thr Met Lys	
170 175 180 185	
CCG GAA CCG AAT GCG TCT GTC GAG GGA TAC TTC AGC AAG CAG	623
Pro Glu Pro Asn Ala Ser Val Glu Gly Tyr Phe Ser Lys Gln	
190 195	
TAAACCTGTG AGCGTCGAG GAGTCAGTGT GACCTCACCC GCCTCTGCCA GTGGGTGCGA	683
GAGGGCGTGA GGGATTGTGG GAAGGCTGTT GGATATGATG CAGACAGCGA TGAATGCAAC	743
TCCACACAC TGGCCCTCCT CAGCCCTCTC CACACAGACA CACGCACGCA TGTG	797

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

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- (A) LENGTH: 199 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

```

Met Ser Cys Gly Asn Ala Lys Ile Asn Ser Pro Ala Pro Ser Phe Glu
 1             5             10             15

Glu Val Ala Leu Met Pro Asn Gly Ser Phe Lys Lys Ile Ser Leu Ser
      20             25             30

Ser Tyr Lys Gly Lys Trp Val Val Leu Phe Phe Tyr Pro Leu Asp Phe
      35             40             45

Ser Phe Val Cys Pro Thr Glu Val Ile Ala Phe Ser Asp Ser Val Ser
      50             55             60

Arg Phe Asn Glu Leu Asn Cys Glu Val Leu Ala Cys Ser Ile Asp Ser
      65             70             75             80

Glu Tyr Ala His Leu Gln Trp Thr Leu Gln Asp Arg Lys Lys Gly Gly
      85             90             95

Leu Gly Thr Met Ala Ile Pro Met Leu Ala Asp Lys Thr Lys Ser Ile
      100            105            110

Ala Arg Ser Tyr Gly Val Leu Glu Glu Ser Gln Gly Val Ala Tyr Arg
      115            120            125

Gly Leu Phe Ile Ile Asp Pro His Gly Met Leu Arg Gln Ile Thr Val
      130            135            140

Asn Asp Met Pro Val Gly Arg Ser Val Glu Glu Val Leu Arg Leu Leu
      145            150            155            160

Glu Ala Phe Gln Phe Val Glu Lys His Gly Glu Val Cys Pro Ala Asn
      165            170            175

Trp Lys Lys Gly Ala Pro Thr Met Lys Pro Glu Pro Asn Ala Ser Val
      180            185            190

Glu Gly Tyr Phe Ser Lys Gln
      195

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(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 637 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Leishmania tropica*

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 7..624

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

TTACAT ATG CAT CAC CAC CAC CAC CAC ATG TCC TGC GGT AAC GCC AAG	48
Met His His His His His His Met Ser Cys Gly Asn Ala Lys	
1 5 10	
ATC AAC TCT CCC GCG CCG CCC TTC GAG GAG ATG GCG CTC ATG CCC AAC	96
Ile Asn Ser Pro Ala Pro Pro Phe Glu Glu Met Ala Leu Met Pro Asn	
15 20 25 30	
GGC AGC TTC AAG AAG ATC AGC CTC TCC GCC TAC AAG GGC AAG TGG GTC	144
Gly Ser Phe Lys Lys Ile Ser Leu Ser Ala Tyr Lys Gly Lys Trp Val	
35 40 45	
GTG CTC TTC TTC TAC CCG CTC GAC TTC ACC TTC GTG TGC CCG ACA GAG	192
Val Leu Phe Phe Tyr Pro Leu Asp Phe Thr Phe Val Cys Pro Thr Glu	
50 55 60	
ATC ATC GCG TTC TCC GAC AAC GTG AGT CGC TTC AAC GAG CTC AAC TGC	240
Ile Ile Ala Phe Ser Asp Asn Val Ser Arg Phe Asn Glu Leu Asn Cys	
65 70 75	
GAG GTC CTC GCG TGC TCG ATG GAC AGC GAG TAC GCG CAC CTG CAG TGG	288
Glu Val Leu Ala Cys Ser Met Asp Ser Glu Tyr Ala His Leu Gln Trp	
80 85 90	
ACG CTG CAG GAC CGC AAG AAG GGC GGC CTC GGG GCC ATG GCG ATC CCA	336
Thr Leu Gln Asp Arg Lys Lys Gly Gly Leu Gly Ala Met Ala Ile Pro	
95 100 105 110	
ATG CTG GCC GAC AAG ACT AAG AGC ATC GCT CGT TCC TAC GGC GTG CTG	384
Met Leu Ala Asp Lys Thr Lys Ser Ile Ala Arg Ser Tyr Gly Val Leu	
115 120 125	
GAG GAG AGC CAG GGC GTG GCC TAC CGC GGT CTC TTC ATC ATC GAC CCC	432
Glu Glu Ser Gln Gly Val Ala Tyr Arg Gly Leu Phe Ile Ile Asp Pro	
130 135 140	
CGT GGC ATG GTG CGT CAG ATC ACC GTC AAC GAC ATG CCG GTG GGC CGC	480
Arg Gly Met Val Arg Gln Ile Thr Val Asn Asp Met Pro Val Gly Arg	
145 150 155	
AAC GTG GAG GAG GCT CTG CGC CTG CTG GAG GCT TTG CAG TTC GTG GAG	528
Asn Val Glu Glu Ala Leu Arg Leu Leu Glu Ala Leu Gln Phe Val Glu	
160 165 170	

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AAG CAC GGC GAG GTG TGC CCC GCG AAC TGG AAG AAG GGC GCC CCC ACG 576
 Lys His Gly Glu Val Cys Pro Ala Asn Trp Lys Lys Gly Ala Pro Thr
 175 180 185 190

 ATG AAG CCG GAA CCG AAG GCG TCT GTC GAG GGA TAC TTC AGC AAG CAG 624
 Met Lys Pro Glu Pro Lys Ala Ser Val Glu Gly Tyr Phe Ser Lys Gln
 195 200 205

 TAAGAATTCC ATG 637

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 206 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Met His His His His His His Met Ser Cys Gly Asn Ala Lys Ile Asn
 1 5 10 15
 Ser Pro Ala Pro Pro Phe Glu Glu Met Ala Leu Met Pro Asn Gly Ser
 20 25 30
 Phe Lys Lys Ile Ser Leu Ser Ala Tyr Lys Gly Lys Trp Val Val Leu
 35 40 45
 Phe Phe Tyr Pro Leu Asp Phe Thr Phe Val Cys Pro Thr Glu Ile Ile
 50 55 60
 Ala Phe Ser Asp Asn Val Ser Arg Phe Asn Glu Leu Asn Cys Glu Val
 65 70 75 80
 Leu Ala Cys Ser Met Asp Ser Glu Tyr Ala His Leu Gln Trp Thr Leu
 85 90 95
 Gln Asp Arg Lys Lys Gly Gly Leu Gly Ala Met Ala Ile Pro Met Leu
 100 105 110
 Ala Asp Lys Thr Lys Ser Ile Ala Arg Ser Tyr Gly Val Leu Glu Glu
 115 120 125
 Ser Gln Gly Val Ala Tyr Arg Gly Leu Phe Ile Ile Asp Pro Arg Gly
 130 135 140
 Met Val Arg Gln Ile Thr Val Asn Asp Met Pro Val Gly Arg Asn Val
 145 150 155 160
 Glu Glu Ala Leu Arg Leu Leu Glu Ala Leu Gln Phe Val Glu Lys His
 165 170 175

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Gly Glu Val Cys Pro Ala Asn Trp Lys Lys Gly Ala Pro Thr Met Lys
180 185 190

Pro Glu Pro Lys Ala Ser Val Glu Gly Tyr Phe Ser Lys Gln
195 200 205

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 51 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "5' PCR primer (MAPS-1-5'His) to simultaneously amplify MAPS-1 cDNA for both L. major and L. tropica while adding 6 His residues to amino terminal end of encoded protein."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

CAATTACATA TGCATCACCA TCACCATCAC ATGTCCTGCG GTAACGCCAA G

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(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 31 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "3' PCR primer (MAPS-1-3'OR1) to simultaneously amplify MAPS-1 cDNA for both L. major and L. tropica."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

CATGGAATTC TTAGTGCTTG CTGAAGTATC C

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(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 520 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Leishmania major

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

GGCACGAGCC CTTGCCTACA TTTGCTCGCC GATATTCGCG GGGAGTTCTT CAATTTGCGT	60
CGCGTAGAAC TGCTCAATGT CGCGCAACAA GCGCAGCTCG TCGTGGCGCA CGAAGGTGAT	120
GGCCAGTCCA GTGCGGCCCA TGCGGCCAGT GCGGCCGATG CGGTGAATGT ACTGCTCACG	180
CGCGAGCGGC AAATCGTAGC TGAGGACGAG CGAGACGCGC TCCACATCAA TGCCACGCGC	240
CCACAGGTCC GTTGTAATGA NCACGCGGCT GTGTCCATTA CGGAATGCCG CATAATCTCG	300
TCGCGCTCCG CCTGGGGCAT GTCGCCGTGC ATGGCGGACA CAGCGAAATT CTCGCGCGTC	360
ATCTTCTTGG CAAGCTGCTC CACCTTTTTG CGGGTGTTC ANAAAACCAC NGCGTGGGCG	420
ATCGTTAAGC TGTCGTACAA ACTCCATCAA GAAATCGAAT TTGTTTTTCT CTTCGTCNAC	480
NGANACAAAN TACTGTTTAA CGCTNTCCAC GGTGATCTCA	520

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 600 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Leishmania major*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GGCACAAGGT TTTCGGGTTA TCTTCACGCA TGGTGGAGCG CAGATGGGTG AAGTAAATAC	60
GCGGACCGAA CTGCTTGATC ATATCAACCA GATCGTTGTC AGCACGCACG CCGTANGAAC	120
CGGTGCACAT GGTAAAACCG TNTGCCATGC TGTTTACGGT ATCAACCATC CACTGCATAT	180
CTTCAATGGT GGAAACAATG CGCGGCAGGC CGAGGATCCG GCGCGGCTCA TCATNNAGNT	240
NATNAACCAN TCGCACGTCT ANTTCTGCAC TAAACTACAA NTATCGGTNA CATATNATAA	300
GGCCNATTTT CGGTCCAGGA NTATGTNCTN TCAAAATGCC NCGTTANNCA CTCTTAAATG	360
TCTCANGNGN AAANTNGTTC TAAAGGGTGT CCAAANNTN NTTACCNTTC CCCNCTTACT	420
TCAANANCTC CTCNAATTCC CNGGCCCTTN GACNANNATT TNCTATTAAA ANATANAANN	480

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TTCAAATTNA TTCCCNACCT NCCNTNNCCA AANNTANCNA ATAATCANNC CCCTNTCANN 540
ANNTCCCAN C TTACCCTCCN NTNGNNGGGN NNNCCNATTN CCCCANCCC NCNCTAAATA 600

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 600 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Leishmania major*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

GGCACGAGCC TCAGTGGAGC TCAATGAAGA TATTGCAGTA TCTTACTCTG GATGGCACTC 60
AGGTCTCCGG CACGCTGCCG CCCAGTGGA GCGCGATGGC ATCGGTGCGA ATTCTTAACC 120
TGNAGGGTAC TGAGGTCTCT GGTACGCTGC CGCCTGAGTG GATATCNATG ANCAGGCTGC 180
AAACTCTGAA TCTGCGGCGC ACGAAANTAT CCGGCACTCT GCCGCCCCGAA TGGANTTCTA 240
TGAACAGCCT GGAGTACTTT CACCTTTATC TTAATCAGGT CTCCGGCACG CTGCCGCCCCG 300
AGTGGAGTGG GATGTCNAAG GCCGCATACT TCTGGCTGGA ATACTGCGAC CTGTCCGGCA 360
NTCTGCCGCC CNAGTGGTCG TCNATGCCAA AGCTGCGCGG TATCTCACTG ANCGGCAACA 420
AATTCTTGCG NGTGTNTNCC NGAATCNTGG GATTCAGAAA GGTGGTCCTT GTTGTGTTGGGC 480
ATCNAAGGAN CAAACCCCAA NGGGCCCN CN AATTGCTTGG GCNTGCTTAA GGANTTGCAC 540
NAACCAACNC CNCCAAAAAC CCCCCCACC NCNAAANNAC NANCCCCAC TTAANNCCCN 600

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 600 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Leishmania major*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

NGCACGAGAA GCGCAACTGG CGCATCGCAT CTGTGACTAT CTGCCTGAAC AGGGGCAATN	60
GTTTGTGGT AACAGCCTGG TGGTACGTCT GATTGATNCG CTTCGCAAN TTCCGGCAGG	120
TTACCCGGTG TACANCAACC GTGGGGCCAN CGGTATCNAC NGGCTGCTTT CGACCGCCGC	180
CGGNGTTCAN CGGGCAANCG GCAAACCGAC GCTGGCGATT GTGGGCGATC TCTCCGCACT	240
TTACGATCTC AACGCNCTGG CGTTATTGCG TCAGGTTTCT GCGCCGCTGG TATTAATTGT	300
GGTGAACAAC AACGGCNGGG CAAAATTTTC TCGCTGTTGC CAACGCCCCC AAAGCNAGCG	360
TGAAGCGTTT CTATCTGATG CCGCAAAACG TCCATTTTGA AACACGCCGC CNCCCATGTT	420
TCGANCTGAA AATATCATCG TCCGCAAAAC TGGCANGAAA CTTNGAAAAC CGCATTTTGC	480
CGACNCCCTG GCNCACGCCC AACCCACCCA CCGGTTGATT GAAAATGGTG GGTAAACGAA	540
NCCNNATGGG TGCCCCAAAN CNCNNCCANC CAAATTTCTG GGCCCAGGTT AAANCCCTTT	600

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 600 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Leishmania major*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

ACGATGACCA TGCCCCGAAG GAGGATGGCC ATGCGCCGAA GAACGATGAC CATGCCCCGA	60
AGGAGGATGG CCATGCGCCG AAGAACGATG ACCATGCCCC GAAGGAGGAT GGCCATGCGC	120
CGAAGAACGA CGGGGATGTG CAGAANAAGA GCGAAGATGG AGACAACGTG GGAGAGGGAG	180
GCAAGGGCAA TGAGGATGGT AACGATGATC AGCCGAAGGA GCACGCTGCC GGCAACTAGT	240
GGGCTGCGTC CGGGCTTGTG TGCGANCCGT GCTCTGCACC CCGCCGCTCG TGCATCCTCG	300
CATGTGGACT GCGTGTGTCT CTCCCGCTTT GTCTCTCTCC CCCACACAGT GGCTGATGCC	360

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TGCACGGGGT TGCTGTGGCT GCACCTCCTG ACCACTGCCA GCTTTCTTGG CTTGCCTCCC	420
CTCTGCGCCT CCGCTCGTGC CGCTCGTGCC GAATTCGATA TCAAGCTTAT CGATACCGTC	480
NACCTCGAAG GGGGGCCCCG TTACCCATTC GCCCTATANT GAGTCNTATT ACAATTCCTG	540
GCGTCGTTTT ACACGTCGTG ACTGGGAAAA ACCCTGGCGT TCCCCACTTA TCGCCTTGCA	600

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 516 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Leishmania major

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

AGCTGCAGCA GCGCCTAGAC ACCGCCACGC AGCAGCGCGC CGAGCTGGAG GCACGGGTGG	60
CACGGCTGGC CGCGGACCGC GACGAGGCGC GCCAGCAGCT GGCCGCGAAC GCCGAGGAGC	120
TGCAGCAGCG CCTAGACACC GCCACGCAGC AGCGCGCCGA GCTGGAGGCA CGGGTGGCAC	180
GGCTGGCCGC GGACGGCGAC GAGGCCCCGCC AGCAGCTGGC CGCGAACGCC GAGGAGCTGC	240
AGCAGCGCCT AGACACCGCC ACGCAGCAGC GCGCCGAGCT GGAGGCACAG GTGGCACGGC	300
TGGCCGCGAA CGCCGAGGAG CTGCAGCAGC GCCTAGACAC CGCCACGCAG CAGCGCGCCG	360
AGCTGGAGGC ACGGGTGGCA CGGCTGGCCG CGGACCGCGA CGAGGCGCGC CAGCAGCTGG	420
CCGCGAACGC CGAGGAGCTG CAGCAGCGCC TAGACACCGC CACGCAGCAG CGCGCCGAGC	480
TGGARGCACA GGTGGCACGG CTGGCCGCGA AMGCCG	516

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 822 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

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(A) ORGANISM: *Leishmania major*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

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GGCACGANAG ATCTTCGTGA AGACGCTGAC CGGCAANACG ATCGCGCTGG AGGTGGAGCC      60
GAGCGACACG ATCGAGAACG TGAAGGCCAA GATCCAGGAC AAGGAGGGCA TCCCGCCGGA      120
CCAGCAGCGC CTGATCTTCG CCGGCAAGCA GCTGGAGGAN GGCCGCACGC TCTCGGACTA      180
CAACATCCAG AAGGAGTCCA CGCTGCACCT GGTGCTGCGC CTGCGCGGCG GCATGCANAT      240
CTTCGTGAAA ACGCTNACCG GCAANACAAT CGCGCTGGAA GTGGAGCCGA ACGACCNATC      300
GAAAACGTGA AGGCCNANAT CCANGACAAG GAAGGCNTCC CGCCGGANCA GCACGCCTGA      360
TCTTCCNCCG GCAACCACTT GANGAAGGGC NCACGCTCTC NGACTIONAC ATCCANAAAG      420
GATTCCNCCC TGCACCTTGT TGCTTGCNCC TTGCTCGGGG GGCATGCCNA ATCTTCCTTN      480
AAAACCTCAA CCGGCAANAA CAATCCCCCN CNGAAGTTGG AACCCAACCA NCCCATTENA      540
AAACTTTAAA GGCCNNNATT CCNGAACAAN GAAGGGCTTC CCCCCCGGAC CNNCAANCNC      600
CCTGATTNTT CCCCCGGNNN NCANTTTGGA ANGAAGGGCC CNCCCTCCN CCGAATTNCN      660
ACNTCCCNAA ANGGATTCCC CCCCTNCCCT TGNTTTTTGC GCCNNNNNNC GGCNNCNTNC      720
CNAAATTCCG NCCNAAGGNC CCCANTANAN CNACTTTCCC NTTCCCCCCC NNNNTTTTGC      780
NTAAANTTTT TNCCCCCNNA AANNTCCCNT TTNCNANTTN AN                        822

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(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 146 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Leishmania major*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

```

Gly Thr Ser Pro Cys Leu His Leu Leu Ala Asp Ile Arg Gly Glu Phe
1           5           10           15

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Phe Asn Leu Arg Arg Val Glu Leu Leu Asn Val Ala Gln Gln Ala Gln

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20 25 30
 Leu Val Val Ala His Glu Gly Asp Gly Gln Ser Ser Ala Ala His Ala
 35 40 45
 Ala Ser Ala Ala Asp Ala Val Asn Val Leu Leu Thr Arg Glu Arg Gln
 50 55 60
 Ile Val Ala Glu Asp Glu Arg Asp Ala Leu His Ile Asn Ala Thr Arg
 65 70 75 80
 Pro Gln Val Arg Cys Asn Xaa His Ala Ala Val Ser Ile Thr Glu Cys
 85 90 95
 Arg Ile Ile Ser Ser Arg Ser Ala Trp Gly Met Ser Pro Cys Met Ala
 100 105 110
 Asp Thr Ala Lys Phe Ser Arg Val Ile Phe Leu Ala Ser Cys Ser Thr
 115 120 125
 Phe Leu Arg Val Leu Xaa Lys Thr Thr Ala Trp Ala Ile Val Lys Leu
 130 135 140
 Ser Tyr
 145

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 77 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Leishmania major*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Ala Gln Gly Phe Arg Val Ile Phe Thr His Gly Gly Ala Gln Met Gly
 1 5 10 15
 Glu Val Asn Thr Arg Thr Glu Leu Leu Asp His Ile Asn Gln Ile Val
 20 25 30
 Val Ser Thr His Ala Val Xaa Thr Gly Ala His Gly Lys Thr Val Cys
 35 40 45
 His Ala Val Tyr Gly Ile Asn His Pro Leu His Ile Phe Asn Gly Gly
 50 55 60

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Asn Asn Ala Arg Gln Ala Glu Asp Pro Ala Arg Leu Ile
 65 70 75

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 68 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Leishmania major*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

His Glu Pro Gln Trp Ser Ser Met Lys Ile Leu Gln Tyr Leu Thr Leu
 1 5 10 15
 Asp Gly Thr Gln Val Ser Gly Thr Leu Pro Pro Gln Trp Ser Ala Met
 20 25 30
 Ala Ser Val Arg Ile Leu Asn Leu Xaa Gly Thr Glu Val Ser Gly Thr
 35 40 45
 Leu Pro Pro Glu Trp Ile Ser Met Xaa Arg Leu Gln Thr Leu Asn Leu
 50 55 60
 Arg Arg Thr Lys
 65

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 65 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Leishmania major*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Ala Arg Glu Ala Gln Leu Ala His Arg Ile Cys Asp Tyr Leu Pro Glu
 1 5 10 15

117

Gln Gly Gln Xaa Phe Val Gly Asn Ser Leu Val Val Arg Leu Ile Asp
 20 25 30

Xaa Leu Xaa Gln Xaa Pro Ala Gly Tyr Pro Val Tyr Xaa Asn Arg Gly
 35 40 45

Ala Xaa Gly Ile Xaa Xaa Leu Leu Ser Thr Ala Ala Gly Val Xaa Arg
 50 55 60

Ala
 65

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 78 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Leishmania major

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Asp Asp His Ala Pro Lys Glu Asp Gly His Ala Pro Lys Asn Asp Asp
 1 5 10 15

His Ala Pro Lys Glu Asp Gly His Ala Pro Lys Asn Asp Asp His Ala
 20 25 30

Pro Lys Glu Asp Gly His Ala Pro Lys Asn Asp Gly Asp Val Gln Xaa
 35 40 45

Lys Ser Glu Asp Gly Asp Asn Val Gly Glu Gly Gly Lys Gly Asn Glu
 50 55 60

Asp Gly Asn Asp Asp Gln Pro Lys Glu His Ala Ala Gly Asn
 65 70 75

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 169 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:

118

(A) ORGANISM: *Leishmania major*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Leu Gln Gln Arg Leu Asp Thr Ala Thr Gln Gln Arg Ala Glu Leu Glu
1 5 10 15

Ala Arg Val Ala Arg Leu Ala Ala Asp Arg Asp Glu Ala Arg Gln Gln
 20 25 30

Leu Ala Ala Asn Ala Glu Glu Leu Gln Gln Arg Leu Asp Thr Ala Thr
 35 40 45

Gln Gln Arg Ala Glu Leu Glu Ala Arg Val Ala Arg Leu Ala Ala Asp
 50 55 60

Gly Asp Glu Ala Arg Gln Gln Leu Ala Ala Asn Ala Glu Glu Leu Gln
65 70 75 80

Gln Arg Leu Asp Thr Ala Thr Gln Gln Arg Ala Glu Leu Glu Ala Gln
 85 90 95

Val Ala Arg Leu Ala Ala Asn Ala Glu Glu Leu Gln Gln Arg Leu Asp
 100 105 110

Thr Ala Thr Gln Gln Arg Ala Glu Leu Glu Ala Arg Val Ala Arg Leu
 115 120 125

Ala Ala Asp Arg Asp Glu Ala Arg Gln Gln Leu Ala Ala Asn Ala Glu
 130 135 140

Glu Leu Gln Gln Arg Leu Asp Thr Ala Thr Gln Gln Arg Ala Glu Leu
145 150 155 160

Glu Ala Gln Val Ala Arg Leu Ala Ala
 165

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 98 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Leishmania major*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

119

Ala Arg Xaa Ile Phe Val Lys Thr Leu Thr Gly Xaa Thr Ile Ala Leu
 1 5 10 15

Glu Val Glu Pro Ser Asp Thr Ile Glu Asn Val Lys Ala Lys Ile Gln
 20 25 30

Asp Lys Glu Gly Ile Pro Pro Asp Gln Gln Arg Leu Ile Phe Ala Gly
 35 40 45

Lys Gln Leu Glu Xaa Gly Arg Thr Leu Ser Asp Tyr Asn Ile Gln Lys
 50 55 60

Glu Ser Thr Leu His Leu Val Leu Arg Leu Arg Gly Gly Met Xaa Ile
 65 70 75 80

Phe Val Lys Thr Leu Thr Gly Xaa Thr Ile Ala Leu Glu Val Glu Pro
 85 90 95

Asn Asp

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 39 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Leishmania major

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Leu Gln Gln Arg Leu Asp Thr Ala Thr Gln Gln Arg Ala Glu Leu Glu
 1 5 10 15

Ala Arg Val Ala Arg Leu Ala Ala Asp Arg Asp Glu Ala Arg Gln Gln
 20 25 30

Leu Ala Ala Asn Ala Glu Glu
 35

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 600 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

120

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Leishmania chagasi*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

CGGCCGCCTC AGCGAGGAGG AGATCGAGCG CATGGTGC GC GAGGCTGCCG AGTTCGAGGA	60
TGAGGACCGC AAGGTGCGCG AACGTGTCTGA AGCGAAGAAC TCGCTAGAGA GCATCGCGTA	120
CTCGCTTCGC AACCAGATCA ACGACAAGGA CAAGCTTGGT GACAAGCTCG CCGCGGACGA	180
CAAGAAGGCG ATCGAGGAGG CTGTGAAGGA TGCCCTCGAC TTTGTCCACG AGAACCCCAA	240
TGCAGACCGT GAGGAGTTCG AGGCTGCTCG CACGAAGCTG CAGAGTGTGA CGAACCCCAT	300
CATTCAAAAG GTGTACCAGG GCGCCGCCGG CTCTGGTGCA GAAGAGGCGG ACGCGATGGA	360
TGACTTGTTA GTCGGCCGCG TGAAAAGAAA AACAGGGAAA GCGGGAACAT NCCACAANAA	420
CCNAAGAAGA AAGGGGGTNG CGACACCGCT CGAACACCGA CGGCNCACAT NCNTCATGGG	480
CATGCTCAGC TTTCTCTCC CCAACAAACC AGAAGGTTTT CTCCAAACNC CGTCTCNGCN	540
CCCAAAATAC GGAAANGTTA ANCGAAAAAN CCCCTTCCAC CAATTGNNGT TCTTTTGTTT	600

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1748 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Leishmania chagasi*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

CTAGTGGATC CCCCGGGCTG CAGGAATTCA CGGAATACGT ACCTCCTCCC CCTTCTTGGT	60
AGAAGAACAA CAACAACGTT CAAGACGACG CCGCGCCTTC TTGTACCGCA TTTGCTTCTG	120
AGCACGTTCA ATCCGTGCCT TGCAAACATG GAGGCGTACA AGAAGCTGGA AACGATCTTT	180
ACGAAGGTCT ACCGCCTGGA CCACTTCCTC GGTCTGGGCA ACTGGGACAT GAACACAAAC	240

ATGCCCCCA AGGGCGAGGA ATCACGCGGT GAGGCGATGG CGATGCTCTC GGAGCTCCGC	300
TTTGGCTTCA TCACGGCACC GGAGGTGAAA AGCCTGATTG AGAGTGCCAC CAAGGGCAGC	360
GAGGAGCTGA ATGCGGTGCA GCGCGCTAAC TTGCGGGAGA TGAGGCGTGC GTGGAAGAGC	420
GCCACCGCCT TGCCGGCTGA GTTTGTGGGC CGCAAGATGC GCCTCACGAC ACACGCGCAC	480
AGCGTGTGGC GCGACAGCCG CAAAGCAAAT GACTTCGCCA AGTTCCTACC GGTGCTCAGG	540
GACCTGGTGG CGCTCGCCCG TGAGGAGGGC TCATACCTCG CCGCCGGCAC CTCCCTCTCC	600
CCGTATGAGG CGCTCATGAA CGAGTACGAG CCAGGAATCA CGACACAAAA GCTGGATGAG	660
GTGTACGCAA ATGTAAAGTC GTGGCTGCCG CAGCTGCTAA AGGACATTGT GCAGAAGCAG	720
TCCGGCGAGT CCGTGATTGC GTTCTCGCAT AAGTTCCCGC AGGACAAGCA GGAAGCACTG	780
TGCAAGGAAT TCATGAAGAT CTGGCACTTC GACACCGATG CCGGTCGCCT CGACGTCAGC	840
CCCCACCCTT TCACGGGAAT GACGAAGGAG GACTGCCGAC TCACAACAAA CTACATCGAA	900
GACACGTTTG TTCAGAGCTT GTATGGCGTC ATCCACGAGA GTGGGCATGG CAAGTACGAG	960
CAGAACTGTG GCCCACGCGA GCACATCACG CAGCCGGTGT GCAACGCCCC CTCTCTTGGC	1020
CTGCATGAGA GCCAGAGCCT CTTTGCGGAG TTTCAGATCG GCCACGCGAC GCCCTTCATC	1080
GA CTACCTCA CAACTCGCCT TCCTGAGTTC TTCGAGGCGC AGCCAGCGTT CTCGCAGGAC	1140
AACATGCGCA AGTCGCTGCA GCAGGTGAAG CCGGGCTACA TTCGCGTCGA TGCCGATGAG	1200
GTGTGCTACC CTCTGCACGT GATCCTGCGC TACGAGATCG AGCGCGACTT GATGGAGGGC	1260
AAAATGGAGG TGGAAGACGT GCCGCGCGCG TGGAACGCAA AGATGCAGGA GTACTTGGGT	1320
CTCTCAACGG AGGGCCGTGA CGACGTTGGG TGCCTGCAGG ACGTGCAATG GTCCATGGTG	1380
CGCTCGGCTA CTCTCCGACG TACTCGCTCG GCGCCATGTA TGCGGCGCAG ATCATGGCGA	1440
GCATCCGAAA GGAGCTGGGA GACGACAAGG TGGATGAGTG CCTGCGCACC GGTGAGCTCG	1500
GCCCCCTCCT GGAAAAGCAG CAGGAGAAGA TCTGGGATCA TGGGTGCCTG TACGAGACGG	1560
ACGACCTCAT GACGCGTGCG ACGGGCGAGA CGCTGAACCC CGAGTACCTG CGCCGCCACC	1620
TGGAGGCGCG CTACATAAAC GCCTGAGTCG CGAGCGGTTG ACACACGCGC TCGCTAGCAC	1680
ATGACGCGTC TTTATTATTC TTTGTTGTGC ATTCGGAATT CCGCGGAATT CGATATCAAG	1740
CTTATCGA	1748

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(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 560 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Leishmania chagasi*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

```

CGGAAGGAGG ATGCCATAC ACAGAAAAT GACGGCGATG GCCCTAAGGA GGACGGCCGT      60
ACACAGAAAA ACGACGACGG TGGCCCTAAG GAGGACGGCC ATACACAGAA AAATGACGGC      120
GATGGCCCTA AGGAGGACGG CCGTACACAG AAAAATAACG GCGATGGCCC TNAGGAGGAC      180
GGCCATACAC AGAAAAATGA CGGCGATGCC CCTNAGGAGG ACGGCCGTAC ACANAAAAAT      240
GACGGCNATG GCCCTNAGGA GGACGGCCGT ACACAGAAAA ATGACNGCCA TGGCCCTTAG      300
GANGACGCCG TACACAGAAA AATGACGCNA TGGCCCTNAG GGAGGACGGC CATACCCANA      360
AAAATTGACG GCNATNGCCC TTAGGANGAC GGCCGTNCCC ANAAANANTG ACNGCGGTNG      420
CCCTTAAGGA AGATGAAAAT CTGCCACCAA AACNATTGGG AATGCNCAGG AAAANAACNA      480
ANATNGACCC CACGTGGGGG ATGGANCTTA CNGCNATTAA NATTGTTACC ATTATCNACC      540
NAAGGACNNG TTGCCGNCAA

```

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 600 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Leishmania chagasi*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

```

CGTCCGAGAA ACCCGTACAT GTATGCTGCT GGTAGAAGGC GCAGAGCTGG TCCCTCTGAT      60
GCACAAGCAT GAGGTCGTAC ATTGCTGGT TCGTCATTTT CCAGAGCACA ACGAGCAGCG      120

```


TCATCATACA GCATCCAATA GCCGCCAGAG TGAATGCGAT GCGCACACCA AGTCGAAAGT	180
GGTCGACCAG TAGGGGAATG TGACCCTGGC TGGCGTGCAA CATGATCGCC ACGCCAGCGG	240
TGGGCCACAC CACAACAGAG GCGACGAAAG AGAACATGAA CTTGCTCACG AAGCTNACAA	300
TAAGGGCGTC GCTNGTGATG CTAAGAACCA CGCCNAGGTA GACGGCGAAG ANCAAATAA	360
ACACAAGCGT GACGATCCCG AAAAGAAGGA TCTCTGCGGA ATTTTCGTGA GATAGANAAT	420
CCCCGTA CTG GAAAAANAAG CCGGCAGGCG CGCGATAACG CTGCAACTTG CCGCTCCTCG	480
CGGGCGCGTT TTCGCTCCTT CTCCGACTTG ATGGCGCNGT CNGNCTTGAC AAAACGGTTA	540
AGCTCCTCAT GCCCCAGCCG ATTCCCAGCT CACGGTCCAC TTCCGGCCAT GCCCACGGAC	600

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1053 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Leishmania chagasi*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

GGGAAAAAAG TGGAGCTCCA CCGCGGTGGC GGCCGCTCTA GAACTAGTGG ATCCCCCGGG	60
CTGCAGGAAT TCCGCGGAAT TCCGCGGAAT TCCGCGGAAT TCCGTCCGAC GCGGCACCCG	120
CACAGGGGTC GACAGTGACG CAACCTCCTC CACCACTGCG GCCTACGACG GCGCCGGCTC	180
CGCGCCAGTG ATGGTTGACG CCAATGTGAG CCACCCTCCG TACGCGGGGC ATGACCAAGT	240
GTACATGCAC GTCGGCAAGC CCATCGTGGG CAACACCCTC GACGGATACA ACGGGTGCGT	300
GTTTCGCTAC GGGCANACGG GCAGCGGCAA AACCTTCACG ATGCTCGGNT ACGCGCCGAG	360
CACGANCGAC ATCCGCGCTC GCAAAGGGTC CGTCCCCTGC GGGGCCAGCA GCATGGAGAA	420
CAGCACTCCT CTTGACAGCG CTGTGGAGCC GTTTGAGAGC GATGACGGCG ACGACGTGGT	480
GGACAAGACG GGGCTGGATC CGAACGAGCT GCAAGGCATC ATCCCGCGCG CGTGACGGGA	540
CCTGTTTCGAT GGTCTCCGTG CGAAGCGCGC CAAGGACTCC GACTTCACGT ACCGCGTGGA	600

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```

GGTGTCTTAC TACGAGATCT ACAACGAGAA GGTGTTCGAT CTCATCCGGC CGCAGCGCAA      660
CACGGACCTG AGGATACGTA ACTCGCCCCA CTCCGGTCCA TTTATCGAAG GCCTGACGTG      720
GAAGATGGTG TCCAAGGAGG AAGACGTCGC CCGCGTGATT CGCAAGGGCA TGCAGGAGCG      780
CCACACGGCT GCGACCAAGT TCAACGACCG CAGCAGCCGC AGCCACGCCA TCCTCACCTT      840
CAACATTGTG CAGCTGTGCA TGGACGACTC CGACAACGCG TTCCAGATGC GCAGCAAGCT      900
GAACCTGGTG GACCTTGCTG GGTCCGAGCG CACTGGTGCG GCCGGAGCCG AGGGCAATGA      960
GTTCCACGAC GGTGTGAAGA TCAACCACTC GCTGACGGTG CTGGGGCGCG TGATCGACCG     1020
TCTGGCGGAC CTCTCGCAGA ACAAGGGAGG GGG                                  1053

```

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 136 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Leishmania chagasi*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

```

Gly Arg Leu Ser Glu Glu Glu Ile Glu Arg Met Val Arg Glu Ala Ala
1           5           10           15

```

```

Glu Phe Glu Asp Glu Asp Arg Lys Val Arg Glu Arg Val Glu Ala Lys
20           25           30

```

```

Asn Ser Leu Glu Ser Ile Ala Tyr Ser Leu Arg Asn Gln Ile Asn Asp
35           40           45

```

```

Lys Asp Lys Leu Gly Asp Lys Leu Ala Ala Asp Asp Lys Lys Ala Ile
50           55           60

```

```

Glu Glu Ala Val Lys Asp Ala Leu Asp Phe Val His Glu Asn Pro Asn
65           70           75           80

```

```

Ala Asp Arg Glu Glu Phe Glu Ala Ala Arg Thr Lys Leu Gln Ser Val
85           90           95

```

```

Thr Asn Pro Ile Ile Gln Lys Val Tyr Gln Gly Ala Ala Gly Ser Gly
100          105          110

```

```

Ala Glu Glu Ala Asp Ala Met Asp Asp Leu Leu Val Gly Arg Val Lys

```

125

115

120

125

Arg Lys Thr Gly Lys Ala Gly Thr
 130 135

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 510 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Leishmania chagasi

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

Tyr Leu Leu Pro Leu Leu Gly Arg Arg Thr Thr Thr Thr Phe Lys Thr
 1 5 10 15

Thr Pro Arg Leu Leu Val Pro His Leu Leu Leu Ser Thr Phe Asn Pro
 20 25 30

Cys Leu Ala Asn Met Glu Ala Tyr Lys Lys Leu Glu Thr Ile Phe Thr
 35 40 45

Lys Val Tyr Arg Leu Asp His Phe Leu Gly Leu Gly Asn Trp Asp Met
 50 55 60

Asn Thr Asn Met Pro Pro Lys Gly Glu Glu Ser Arg Gly Glu Ala Met
 65 70 75 80

Ala Met Leu Ser Glu Leu Arg Phe Gly Phe Ile Thr Ala Pro Glu Val
 85 90 95

Lys Ser Leu Ile Glu Ser Ala Thr Lys Gly Ser Glu Glu Leu Asn Ala
 100 105 110

Val Gln Arg Ala Asn Leu Arg Glu Met Arg Arg Ala Trp Lys Ser Ala
 115 120 125

Thr Ala Leu Pro Ala Glu Phe Val Gly Arg Lys Met Arg Leu Thr Thr
 130 135 140

His Ala His Ser Val Trp Arg Asp Ser Arg Lys Ala Asn Asp Phe Ala
 145 150 155 160

Lys Phe Leu Pro Val Leu Arg Asp Leu Val Ala Leu Ala Arg Glu Glu
 165 170 175

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Gly Ser Tyr Leu Ala Ala Gly Thr Ser Leu Ser Pro Tyr Glu Ala Leu
 180 185 190

Met Asn Glu Tyr Glu Pro Gly Ile Thr Thr Gln Lys Leu Asp Glu Val
 195 200 205

Tyr Ala Asn Val Lys Ser Trp Leu Pro Gln Leu Leu Lys Asp Ile Val
 210 215 220

Gln Lys Gln Ser Gly Glu Ser Val Ile Ala Phe Ser His Lys Phe Pro
 225 230 235 240

Gln Asp Lys Gln Glu Ala Leu Cys Lys Glu Phe Met Lys Ile Trp His
 245 250 255

Phe Asp Thr Asp Ala Gly Arg Leu Asp Val Ser Pro His Pro Phe Thr
 260 265 270

Gly Met Thr Lys Glu Asp Cys Arg Leu Thr Thr Asn Tyr Ile Glu Asp
 275 280 285

Thr Phe Val Gln Ser Leu Tyr Gly Val Ile His Glu Ser Gly His Gly
 290 295 300

Lys Tyr Glu Gln Asn Cys Gly Pro Arg Glu His Ile Thr Gln Pro Val
 305 310 315 320

Cys Asn Ala Arg Ser Leu Gly Leu His Glu Ser Gln Ser Leu Phe Ala
 325 330 335

Glu Phe Gln Ile Gly His Ala Thr Pro Phe Ile Asp Tyr Leu Thr Thr
 340 345 350

Arg Leu Pro Glu Phe Phe Glu Ala Gln Pro Ala Phe Ser Gln Asp Asn
 355 360 365

Met Arg Lys Ser Leu Gln Gln Val Lys Pro Gly Tyr Ile Arg Val Asp
 370 375 380

Ala Asp Glu Val Cys Tyr Pro Leu His Val Ile Leu Arg Tyr Glu Ile
 385 390 395 400

Glu Arg Asp Leu Met Glu Gly Lys Met Glu Val Glu Asp Val Pro Arg
 405 410 415

Ala Trp Asn Ala Lys Met Gln Glu Tyr Leu Gly Leu Ser Thr Glu Gly
 420 425 430

Arg Asp Asp Val Gly Cys Leu Gln Asp Val His Trp Ser Met Val Arg
 435 440 445

Ser Ala Thr Leu Arg Arg Thr Arg Ser Ala Pro Cys Met Arg Arg Arg
 450 455 460

Ser Trp Arg Ala Ser Glu Arg Ser Trp Glu Thr Thr Arg Trp Met Ser

127

465 470 475 480

Ala Cys Ala Pro Val Ser Ser Ala Pro Ser Trp Lys Ser Ser Arg Arg

 485 490 495

Arg Ser Gly Ile Met Gly Ala Cys Thr Arg Arg Thr Thr Ser

 500 505 510

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 107 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Leishmania chagasi*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

Gly Arg Arg Met Ala Ile His Arg Lys Met Thr Ala Met Ala Leu Arg
1 5 10 15

Arg Thr Ala Val His Arg Lys Thr Thr Thr Val Ala Leu Arg Arg Thr
 20 25 30

Ala Ile His Arg Lys Met Thr Ala Met Ala Leu Arg Arg Thr Ala Val
 35 40 45

His Arg Lys Ile Thr Ala Met Ala Leu Arg Arg Thr Ala Ile His Arg
 50 55 60

Lys Met Thr Ala Met Pro Leu Arg Arg Thr Ala Val His Xaa Lys Met
65 70 75 80

Thr Ala Met Ala Leu Arg Arg Thr Ala Val His Arg Lys Met Thr Ala
 85 90 95

Met Ala Leu Arg Xaa Thr Pro Tyr Thr Glu Lys
 100 105

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 63 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

128

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Leishmania chagasi*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

```

Val Arg Glu Thr Arg Thr Cys Met Leu Leu Val Glu Gly Ala Glu Leu
1           5           10           15
Val Pro Leu Met His Lys His Glu Val Val His Cys Leu Val Arg His
20           25           30
Phe Pro Glu His Asn Glu Gln Arg His His Thr Ala Ser Asn Ser Arg
35           40           45
Gln Ser Glu Cys Asp Ala His Thr Lys Ser Lys Val Val Asp Gln
50           55           60

```

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 324 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Leishmania chagasi*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

```

Phe Arg Gly Ile Pro Arg Asn Ser Val Arg Arg Gly Thr Arg Thr Gly
1           5           10           15
Val Asp Ser Asp Ala Thr Ser Ser Thr Thr Ala Ala Tyr Asp Gly Ala
20           25           30
Gly Ser Ala Pro Val Met Val Asp Ala Asn Val Ser His Pro Pro Tyr
35           40           45
Ala Gly His Asp Gln Val Tyr Met His Val Gly Lys Pro Ile Val Gly
50           55           60
Asn Thr Leu Asp Gly Tyr Asn Gly Cys Val Phe Ala Tyr Gly Xaa Thr
65           70           75           80
Gly Ser Gly Lys Thr Phe Thr Met Leu Gly Tyr Ala Pro Ser Thr Xaa
85           90           95

```

129

Asp	Ile	Arg	Ala	Arg	Lys	Gly	Ser	Val	Pro	Cys	Gly	Ala	Ser	Ser	Met
			100					105					110		
Glu	Asn	Ser	Thr	Pro	Leu	Asp	Ser	Ala	Val	Glu	Pro	Phe	Glu	Ser	Asp
		115					120					125			
Asp	Gly	Asp	Asp	Val	Val	Asp	Lys	Thr	Gly	Leu	Asp	Pro	Asn	Glu	Leu
	130						135					140			
Gln	Gly	Ile	Ile	Pro	Arg	Ala	Cys	Thr	Asp	Leu	Phe	Asp	Gly	Leu	Arg
145					150					155					160
Ala	Lys	Arg	Ala	Lys	Asp	Ser	Asp	Phe	Thr	Tyr	Arg	Val	Glu	Val	Ser
				165					170					175	
Tyr	Tyr	Glu	Ile	Tyr	Asn	Glu	Lys	Val	Phe	Asp	Leu	Ile	Arg	Pro	Gln
			180					185					190		
Arg	Asn	Thr	Asp	Leu	Arg	Ile	Arg	Asn	Ser	Pro	Asn	Ser	Gly	Pro	Phe
		195						200					205		
Ile	Glu	Gly	Leu	Thr	Trp	Lys	Met	Val	Ser	Lys	Glu	Glu	Asp	Val	Ala
	210						215					220			
Arg	Val	Ile	Arg	Lys	Gly	Met	Gln	Glu	Arg	His	Thr	Ala	Ala	Thr	Lys
225					230					235					240
Phe	Asn	Asp	Arg	Ser	Ser	Arg	Ser	His	Ala	Ile	Leu	Thr	Phe	Asn	Ile
				245					250					255	
Val	Gln	Leu	Ser	Met	Asp	Asp	Ser	Asp	Asn	Ala	Phe	Gln	Met	Arg	Ser
		260						265					270		
Lys	Leu	Asn	Leu	Val	Asp	Leu	Ala	Gly	Ser	Glu	Arg	Thr	Gly	Ala	Ala
		275					280						285		
Gly	Ala	Glu	Gly	Asn	Glu	Phe	His	Asp	Gly	Val	Lys	Ile	Asn	His	Ser
	290					295					300				
Leu	Thr	Val	Leu	Gly	Arg	Val	Ile	Asp	Arg	Leu	Ala	Asp	Leu	Ser	Gln
305					310					315					320
Asn	Lys	Gly	Gly												

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1585 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

AAAGCTGGAG CTCCACCGCG GTGGCGGCCG CTCTAGAACT AGTGGATCCC CCGGGCTGCA	60
GGAATTCGGC ACGAGTGCTG CCCGACATGA CATGCTCGCT GACCGGACTT CAGTGCACAG	120
ACCCGAACTG CAAGACCTGC ACAACTTACG GTCAGTGCAC AGACTGCAAC GACGGCTACG	180
GTCTCACCTC CTCCAGCGTT TGCCTGCGCT GCAGTGTAGC GGGCTGCAAG AGCTGCCCCG	240
TCGACGCTAA CGTCTGCAAA GTGTGTCTCG GCGGCAGCGA GCCGATCAAC AATATGTGCC	300
CCTGCACCGA CCCCAACTGC GCCAGCTGCC CCAGCGACGC TGGCACGTGC ACTCAGTGCG	360
CGAACGGCTA CGGTCTCGTG GACGGCGCCT GTGTGAGATG CCAGGAGCCC AACTGCTTCA	420
GCTGCGACAG CGACGCGAAT AAGTGCACAC AATGTGCGCC GAACTACTAC CTCACCCCGC	480
TCTTGACCTG CTCCCCGGTG GCCTGCAACA TCGAGCACTG CATGCAGTGC GACCCACAGA	540
CGCCGTGCGG CTGCCAGGAG TGCCTGTCCC CCTACGTGGT TGACAGCTAC GACGGCCTCT	600
GCAGGCTCTC CGATGCCTGC TCCGTGCCCA ACTGCAAGAA GTGCGAGACC GGTACCTCCA	660
GGCTCTGCGC CGAGTGCAGC ACCGGCTACA GTCTCTCCGC CGACGCGACG AGCTGCAGCA	720
GTCCAACCAC GCAGCCGTGC GAGGTGGAGC ACTGCAACAC ATGTGTGAAC GGCGATAGCA	780
CCCGCTGTGC CTACTGCAAC ACCGGCTACT ACGTCTCCGA TGGCAAGTGC AAGGCCATGC	840
AGGGCTGCTA CGTGTGCAAC TGCCTGCGAGT GCATGCTGCT TGACAGCACC AAGTGCTCCA	900
CGTGCGTGAA AGGGTACCTG CTCACGTCGT CCTACAGTTG CGTCTCGCAG AAAGTCATCA	960
ACAGTGCGGC CGCGCCCTAC TCTCTGTGGG TGGCCGCCGC CGTGCTCCTC ACCTCTTTTG	1020
CCATGCACCT AGCATAGTGC GCAGCGGCAT GCGAACAACC CCACTCTCAT TCTCCAACAT	1080
GTGCATACAC ACACACACAG ACAGCGGGGC AGCACCCCTT CCCACACAC ACACACGCAC	1140
TTCCCCCTTG TCTTGTCTT CTTTCCTCGN TTCGCATTTC TTTCTCTCGT GCGCTGGCGC	1200
CGGCCTCCTG CACGTCGCTC CCCTCCCCCT AACCTCTATT CTCTCTCTCT CTCTCTCTCG	1260
CCGGCATCAT TGCTTCTTAC CCTTTTCTGA TCCTTGCTCG CGTGGGCGGA CACTGCCACA	1320
GTCCCACAGC GCAGACACAC GTGTTTAAAC GGCGCAGGCA TCCCTCCCTA TCACTTCATT	1380
TCTCCTAAAG CCACTCACCA AGTCGCACAC CGCCCTCCCC CATCGGCCGC CCTTCCGGGC	1440
GCAGCTGTGC GGAATGGGTG TGTGCTCGAC CTCGTTCCTG GCAGCTCACT CGCATGTGTA	1500

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CAGCCACTCC AACCACGAAA GCTCTCTTCT GCGCACATAA AAAAAAAAAA AAAAAAAAAA 1560

CTCGAGGGGG GGCCCGGTAC CCAA 1585

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 320 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

Val	Leu	Pro	Asp	Met	Thr	Cys	Ser	Leu	Thr	Gly	Leu	Gln	Cys	Thr	Asp
1				5					10					15	

Pro	Asn	Cys	Lys	Thr	Cys	Thr	Thr	Tyr	Gly	Gln	Cys	Thr	Asp	Cys	Asn
			20					25					30		

Asp	Gly	Tyr	Gly	Leu	Thr	Ser	Ser	Ser	Val	Cys	Val	Arg	Cys	Ser	Val
	35						40					45			

Ala	Gly	Cys	Lys	Ser	Cys	Pro	Val	Asp	Ala	Asn	Val	Cys	Lys	Val	Cys
	50					55					60				

Leu	Gly	Gly	Ser	Glu	Pro	Ile	Asn	Asn	Met	Cys	Pro	Cys	Thr	Asp	Pro
65					70					75				80	

Asn	Cys	Ala	Ser	Cys	Pro	Ser	Asp	Ala	Gly	Thr	Cys	Thr	Gln	Cys	Ala
			85						90					95	

Asn	Gly	Tyr	Gly	Leu	Val	Asp	Gly	Ala	Cys	Val	Arg	Cys	Gln	Glu	Pro
			100					105						110	

Asn	Cys	Phe	Ser	Cys	Asp	Ser	Asp	Ala	Asn	Lys	Cys	Thr	Gln	Cys	Ala
		115					120					125			

Pro	Asn	Tyr	Tyr	Leu	Thr	Pro	Leu	Leu	Thr	Cys	Ser	Pro	Val	Ala	Cys
	130					135					140				

Asn	Ile	Glu	His	Cys	Met	Gln	Cys	Asp	Pro	Gln	Thr	Pro	Ser	Arg	Cys
145					150					155					160

Gln	Glu	Cys	Val	Ser	Pro	Tyr	Val	Val	Asp	Ser	Tyr	Asp	Gly	Leu	Cys
			165						170						175

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Arg	Leu	Ser	Asp	Ala	Cys	Ser	Val	Pro	Asn	Cys	Lys	Lys	Cys	Glu	Thr
			180					185					190		
Gly	Thr	Ser	Arg	Leu	Cys	Ala	Glu	Cys	Asp	Thr	Gly	Tyr	Ser	Leu	Ser
		195					200					205			
Ala	Asp	Ala	Thr	Ser	Cys	Ser	Ser	Pro	Thr	Thr	Gln	Pro	Cys	Glu	Val
	210					215					220				
Glu	His	Cys	Asn	Thr	Cys	Val	Asn	Gly	Asp	Ser	Thr	Arg	Cys	Ala	Tyr
225					230					235					240
Cys	Asn	Thr	Gly	Tyr	Tyr	Val	Ser	Asp	Gly	Lys	Cys	Lys	Ala	Met	Gln
				245					250					255	
Gly	Cys	Tyr	Val	Ser	Asn	Cys	Ala	Gln	Cys	Met	Leu	Leu	Asp	Ser	Thr
			260					265					270		
Lys	Cys	Ser	Thr	Cys	Val	Lys	Gly	Tyr	Leu	Leu	Thr	Ser	Ser	Tyr	Ser
		275					280					285			
Cys	Val	Ser	Gln	Lys	Val	Ile	Asn	Ser	Ala	Ala	Ala	Pro	Tyr	Ser	Leu
	290					295					300				
Trp	Val	Ala	Ala	Ala	Val	Leu	Leu	Thr	Ser	Phe	Ala	Met	His	Leu	Ala
305					310					315					320

(2) INFORMATION FOR SEQ ID NO:56:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 14 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

Pro	Lys	Glu	Asp	Gly	His	Ala	Pro	Lys	Asn	Asp	Asp	His	Ala
1				5					10				

(2) INFORMATION FOR SEQ ID NO:57:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 7 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

Pro Lys Glu Asp Gly His Ala
1 5

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

Pro Lys Asn Asp Asp His Ala
1 5

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 264 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

ATGCACCATC ATCACCATCA CATGGAAGC TCCTGCACGA AGGACTCCGC AAAGGAGCCC	60
CAGAAGCGTG CTGATAACAT CGATACGACC ACTCGAAGCG ATGAGAAGGA CGGCATCCAT	120
GTCCAGGAGA GCGCCGGTCC TGTGCAGGAG AACTTCGGGG ATGCGCAGGA GAAGAACGAA	180
GATGGACACA ACGTGGGGGA TGGAGCTAAC GACAATGAGG ATGGTAACGA TGATCAGCCG	240

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AAGGAGCAGG TTGCCGGCAA CTAG

264

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 744 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

ATGGGAGCCT ACTGCACGAA GGA	CTCCGCA AAGGAGCCCC	AGAAGCGTGC TGATAACATC	60
CATAAAACCA CTGAGGCCAA TCACAGAGGC	GCCGCCCGTG TGCCCCCGAA	GCACGCCGGC	120
GGTGCGATGA ACGACTCTGC CCCGAAGGAG	GATGGCCATA CACAGAAAAA	TGACGGCGAT	180
GGCCCTAAGG AGGACGGCCG TACACAGAAA	AACGACGACG GTGGCCCTAA	GGAGGACGGC	240
CATACACAGA AAAATGACGG CGATGGCCCT	AAGGAGGACG GCCGTACACA	GAAAAATAAC	300
GGCGATGGCC CTAAGGAGGA CGGCCATACA	CAGAAAAATG ACGGCGATGC	CCCTAAGGAG	360
GACGGCCGTA CACAGAAAAA TGACGGCGAT	GGCCCTAAGG AGGACGGCCG	TACACAGAAA	420
AATGACGGCG ATGGCCCTAA GGAGGACGGC	CGTACACAGA AAAATGACGG	CGATGGCCCT	480
AAGGAGGACG GCCGTACACA GAAAAATGAC	GGCGATGGCC CTAAGGAGGA	CGGCCATACA	540
CAGAAAAATG ACGGCGATGG CCCTAAGGAG	GACGGCCGTA CACAGAAAAA	TGACGGCGGT	600
GGCCCTAAGG AGGATGAGAA TCTGCAGCAA	AACGATGGGA ATGCGCAGGA	GAAGAACGAA	660
GATGGACACA ACGTGGGGGA TGGAGCTAAC	GGCAATGAGG ATGGTAACGA	TGATCAGCCG	720
AAGGAGCAGG TTGCCGGCAA CTAG			744

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 80 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

135

Met Gly Ser Ser Cys Thr Lys Asp Ser Ala Lys Glu Pro Gln Lys Arg
 1 5 10 15
 Ala Asp Asn Ile Asp Thr Thr Thr Arg Ser Asp Glu Lys Asp Gly Ile
 20 25 30
 His Val Gln Glu Ser Ala Gly Pro Val Gln Glu Asn Phe Gly Asp Ala
 35 40 45
 Gln Glu Lys Asn Glu Asp Gly His Asn Val Gly Asp Gly Ala Asn Asp
 50 55 60
 Asn Glu Asp Gly Asn Asp Asp Gln Pro Lys Glu Gln Val Ala Gly Asn
 65 70 75 80

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 247 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

Met Gly Ala Tyr Cys Thr Lys Asp Ser Ala Lys Glu Pro Gln Lys Arg
 1 5 10 15
 Ala Asp Asn Ile His Lys Thr Thr Glu Ala Asn His Arg Gly Ala Ala
 20 25 30
 Gly Val Pro Pro Lys His Ala Gly Gly Ala Met Asn Asp Ser Ala Pro
 35 40 45
 Lys Glu Asp Gly His Thr Gln Lys Asn Asp Gly Asp Gly Pro Lys Glu
 50 55 60
 Asp Gly Arg Thr Gln Lys Asn Asp Asp Gly Gly Pro Lys Glu Asp Gly
 65 70 75 80
 His Thr Gln Lys Asn Asp Gly Asp Gly Pro Lys Glu Asp Gly Arg Thr
 85 90 95
 Gln Lys Asn Asn Gly Asp Gly Pro Lys Glu Asp Gly His Thr Gln Lys
 100 105 110
 Asn Asp Gly Asp Ala Pro Lys Glu Asp Gly Arg Thr Gln Lys Asn Asp
 115 120 125

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Gly Asp Gly Pro Lys Glu Asp Gly Arg Thr Gln Lys Asn Asp Gly Asp
 130 135 140

Gly Pro Lys Glu Asp Gly Arg Thr Gln Lys Asn Asp Gly Asp Gly Pro
 145 150 155 160

Lys Glu Asp Gly Arg Thr Gln Lys Asn Asp Gly Asp Gly Pro Lys Glu
 165 170 175

Asp Gly His Thr Gln Lys Asn Asp Gly Asp Gly Pro Lys Glu Asp Gly
 180 185 190

Arg Thr Gln Lys Asn Asp Gly Gly Gly Pro Lys Glu Asp Gly Asn Leu
 195 200 205

Gln Gln Asn Asp Gly Asn Ala Gln Glu Lys Asn Glu Asp Gly His Asn
 210 215 220

Val Gly Asp Gly Ala Asn Gly Asn Glu Asp Gly Asn Asp Asp Gln Pro
 225 230 235 240

Lys Glu Gln Val Ala Gly Asn
 245

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 6
- (D) OTHER INFORMATION: /note= "Xaa can be either His or

Arg"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 12
- (D) OTHER INFORMATION: /note= "Xaa can be either Gly or

Asp"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 13
- (D) OTHER INFORMATION: /note= "Xaa can be either Asp or

Gly"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

137

Pro Lys Glu Asp Gly Xaa Thr Gln Lys Asn Asp Xaa Xaa Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
(B) LOCATION: 6
(D) OTHER INFORMATION: /note= "Xaa can be either His or

Arg "

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

Pro Lys Glu Asp Gly Xaa Thr
1 5

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
(B) LOCATION: 5
(D) OTHER INFORMATION: /note= "Xaa can be either Gly or

Asp "

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
(B) LOCATION: 6
(D) OTHER INFORMATION: /note= "Xaa can be either Asp or

Gly"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

Gln Lys Asn Asp Xaa Xaa Gly
1 5

(2) INFORMATION FOR SEQ ID NO:66:

138

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 17 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

Gly Cys Gly Pro Lys Glu Asp Gly Arg Thr Gln Lys Asn Asp Gly Asp
 1 5 10 15

Gly

(2) INFORMATION FOR SEQ ID NO:67:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 31 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

Gly Cys Gly Pro Lys Glu Asp Gly Arg Thr Gln Lys Asn Asp Gly Asp
 1 5 10 15

Gly Pro Lys Glu Asp Gly Arg Thr Gln Lys Asn Asp Gly Asp Gly
 20 25 30

(2) INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 45 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

Gly Cys Gly Pro Lys Glu Asp Gly Arg Thr Gln Lys Asn Asp Gly Asp
 1 5 10 15

139

Gly Pro Lys Glu Asp Gly Arg Thr Gln Lys Asn Asp Gly Asp Gly Pro
 20 25 30

Lys Glu Asp Gly Arg Thr Gln Lys Asn Asp Gly Asp Gly
 35 40 45

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 17 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

Gly Cys Gly Pro Lys Glu Asp Gly His Thr Gln Lys Asn Asp Gly Asp
 1 5 10 15

Gly

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 31 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

Gly Cys Gly Pro Lys Glu Asp Gly His Thr Gln Lys Asn Asp Gly Asp
 1 5 10 15

Gly Pro Lys Glu Asp Gly His Thr Gln Lys Asn Asp Gly Asp Gly
 20 25 30

(2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 45 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

140

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

Gly	Cys	Gly	Pro	Lys	Glu	Asp	Gly	His	Thr	Gln	Lys	Asn	Asp	Gly	Asp
1				5					10					15	
Gly	Pro	Lys	Glu	Asp	Gly	His	Thr	Gln	Lys	Asn	Asp	Gly	Asp	Gly	Pro
			20					25					30		
Lys	Glu	Asp	Gly	His	Thr	Gln	Lys	Asn	Asp	Gly	Asp	Gly			
	35						40					45			

(2) INFORMATION FOR SEQ ID NO:72:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 664 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

GCTGCAGGAA TTCGGCACGA GATTGCTTCC CAGCCACCT TCGCTATCCA GCCACTCTCG	60
CTCTTCTACA TCTCCACCCC CCTCACACCG CCATGGCTTC TTCCCGCAAG GCTTCCAACC	120
CGCACAAAGTC GCACCGCAAG CCGAAGCGCT CGTGGAACGT GTACGTGGGC CGCTCGCTGA	180
AGGCGATCAA CGCCAGATG TCGATGTCGC ACCGCACGAT GAAGATCGTG AACTCGTACG	240
TGAACGACGT GATGGAGCGC ATCTGCACTG AGGCCGCGTC GATTGTTGCG CGGAACAAGA	300
AGCGCACGTT GGGTGCGCGC GAGGTGCAGA CGGCGGTGCG CATTGTGCTG CCGGCGGAGC	360
TCGCGAAGCA TGCCATGGCT GAGGGCACGA AGGCCGTGTC GAGCGCGTCC CGCTAAAGCG	420
GCTTGCCGGA TGCCGTGTGA GTAGGAGGGT GGCTTGCCGC AAACGCTGAC CTCGGCGATT	480
GCGGCGTGGC GCTCCCCTTC TCCTCCTTGT CCGGCGGTGT GTGTCATGCA TTTGCGTGAC	540
TCCTCCCTCT TATAGATGCA AGCTTTTTTT TTCTCTTGAC GTTTTATTTT CTCCTCCCCC	600
TCCCTTAACG TGAAGTGAT ATGANAGCGT ACTGGACATG ANANAAAAA AAAANAACT	660
CGAG	664

(2) INFORMATION FOR SEQ ID NO:73:

(i) SEQUENCE CHARACTERISTICS:

141

- (A) LENGTH: 1432 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

GATGAAGAAG AGGAGGACAC CACCATCAAC AACTCCGACG TGGTGGTGCG CTACAAGAAG	60
GCCGCAACGT GGTGCAATGA AACGTTGCGC GTGCTTATCG ATGCCACAAA ACCTGGCGCC	120
AAGGTGTGCG ACCTGTGCCG CCTCGGTGAT GACACCATCA CCGCCNAGGT CAAGACAATG	180
TTCAAAGGCA CGGAAAAAGG CATCGCTTTC CCGACCTGCA TCTCGGTCAA CAACTGCGTA	240
TGCCACAACA GCCCTGGCGT GTCGGACGAG ACGACGCAGC AAGAGATCGC GATGGGTGAC	300
GTCGTGCACT ACGACCTGGG CATCCACGTG GACGGCTACT GCGCCGTCGT CGCGCACACC	360
ATTCAGGTGA CAGAGGACAA TGAGCTTGGC AAGGACGAGA AGGCGGCGCG CGTCATTACA	420
GCGGCGTACA ACATCCTGAA CACGGCGCTG CGCCAGATGC GTCCCGGTAC GACCATCTAC	480
CAGGTGACAG ACGTAGTTGA GAAGGCTGCG GAGCACTACA AGGTGACTCC GGTAGACGGC	540
GTCCTCTCGC ATATGATGAA GCGCTACATC ATAGACNGAT ACCGCTGTAT CCCGCAGCGC	600
AGGGTCGCGG AGCACATGGT GCACGACTAC GATCTCGAGA AAGCGCAGGT GTGGACGCTA	660
GACATTGTCA TGACCTCCGG CAAGGGCAAG CTGAAGGAGC GCGATGCGCG GCCGTGCGTG	720
TTCAAGGTGG CTCTGGACTC CAACTACTCT GTGAAAATGG AAAGCGCGAA GGAGGTTGAG	780
AAGGAAATCG ACTCCNAGTA TGCCACCTTC CCCTTTGCCA TCCGCAACCT GGAGGCCAAG	840
AAGGCCCCGC TCGGTCTCAA CGAGATGGCG AAGCACGGTG CTGTCATCCC GTACCCTATT	900
CTCTTCGAAA AGGAAGGCGA GGTGTCGCGC CATTTCGAAGA TTACGGTGCT CATCAGCAAC	960
AAGAAGATTG AGCCGATTAC CGGCCTGAAG CCGCAGAAGG CCCCGGCGCT CGAGCCATAC	1020
ACGGACGAGA TGCTGCTTGC GACGAACAAG CTCTTCGCTG TCGCTAGAGA AGAAGGCGGC	1080
GAAGTAGACG GCCGTGGCAT CCGTGACGCT GTACTGCGAG CTTTCGTAGG CGTACGCCTC	1140
TTGTGAGGCG TACACGTGTG CTGTTTGCGG ACGAGGAGGC ACCCATTCTG TTCCCCTTCT	1200
TCGCTAATCT TCGCGTTTCC TCTGACGCTG GCTTCTYTGC CGGAGTGTGG TGAGGCGCGT	1260
GGGGGAGAAA CGGCCCACTY GCATGCCTGT GCATACGCGA GCACGGTAGG GAGCGCGGTG	1320

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TGTGTGTGTG TGGGGGGGCG TGTTACGAGT ACAAAGAGG CTCGATCTTT GCGATCTTTT 1380
 CTTTCTGTAA ACAGGAACAT AAGTAACCAA AAAAAAAAAA AAAAAACTCG AG 1432

(2) INFORMATION FOR SEQ ID NO:74:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 873 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

CTTTATTGTC ATCACTGTAA AGCACTGTTT TTTCTTTCAC TTTTCTTGA GTGTTTCTT 60
 CTATTCACCA TGAGCATTAT CAAGGAGGAC GACGCCGTGG GCTGCTACAT GACGGTGACC 120
 CTCGTGGACG ACACCAAGGT GGAGGGTACC ATCTTCACCT ACAATTCCAA GGAGGGCATC 180
 ATAGTACTCC TGTCCCTCCG CGACGATCAG ACGAACATGA AGCTAATCCG CACTCCGTAC 240
 ATCAAAGACT TCAGCCTTTC ACACGCTGAG GAGGGAGCGC ACCTGCCCCC GGCCTGGAC 300
 TCCTTCAACG AGCTTCCGTC CATGCACGCC GGCCGCGACA AGTCCATCTT CAAGCACGCC 360
 AGCACGCAGC TCAAGAACGC CGAGGCGAAC CGCGAAAAGC ACTTCAACTC TGTCACGACC 420
 GACACACCGA TTGCCCACT TGATGCGTAC CTCAAGCTCC TCGCGCTATA CCCCTTAATT 480
 GAGTGGAACA GCGACGAGGG TGTCAATCCAG GTCTCGGACA CCGTCATTGT CGTAGGAGAC 540
 CCCGACTGGC GGACGCCCAA GGCAATGCTG GTGGACGGCG CCCCTGAGAA GGACAGACCG 600
 CTTGTAGATC GCCTGCAGGT TCGGCTCGGM AACGGCAAGA AGTGATTGAG TGTGTAGCGG 660
 ACAGAACATC GTGTGCTTGT GTGTCTGTTT GANGTTTGTT TGTTTTCTCT TTGTGGTACT 720
 GCGTACGACG GCGCCTTCTC CCGGTGGTGG GTGAGTCCAT AAGCAGTTGA GTTCTYGGTT 780
 GTAGNAAVGC CTYACYGCCG ACCATATGGG AGAGGGCGAA CAAATNTTTG ATAGAAGTTG 840
 AAAATCCCAA AGTYAAAAGA AAAAAAAAAAN AAA 873

(2) INFORMATION FOR SEQ ID NO:75:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1238 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

TTTCTGTACT TTATTGAACA TCAGTAGAAC ACGTTCTTCC CGCAAAGATG GCCAAGAAGC	60
ACCTCAAGCG CTTGTATGCG CCCAAGGACT GGATGCTGAG CAAGCTGACC GGCGTGTTTCG	120
CGCCGCGTCC GCGTCCGGGT CCGCACAAAGC TGC GCGAGTG CCTGCCGCTN CTGGTGATCA	180
TCCGCAACCG GCTGAAGTAC GCGCTGAACG CGCGCGAGGG TGAGATGATC CTGCGCCAGG	240
GTCTGGTGCA CGTGGACAAC CACCCGCGCC GCGACGGCAA GTATCCCGCC GGTTTCATGG	300
ACGTGGTCGA GATCCCGAAG ACGGGCGACC GCTTCCGCCT GATGTACGAC GTCAAGGGCC	360
GCTTCGCGTT GGTGAACCTG TCCGAGGCGG AGGCGCAGAT CAAGCTGATG AAGGTTGTGA	420
ACCTGTACAC GGCCACCGGC CGCGTGCCGG TCGCTGTGAC GCACGACGGC CACCGCATCC	480
GCTACCCGGA CCCGCACACC TCCATTGGTG ACACCATCGT GTACAACGTC AAGGAGAAGA	540
AGTGCGTGGA CCTGATCAAG AACCGCCAGG GCAAGGCCGT GATCGTGACC GGTGGCGCCA	600
ACCGCGGCCG CATCGGCGAG ATCGTGAAGG TGGAGTGCCA CCCCGGTGCG TTCAACATTG	660
CGCACCTGAA GGACGCGTCC GCGCGCGAGT TCGCCACCCG CGCCGCGAAC ATCTTCGTGA	720
TCGGCAAGGA CCTGAACAAC CTGCAGGTAA CCGTGCCGAA GCAGCAGGGC CTGCGCATGA	780
ACGTGATCCA GGAGCGCGAG GAGCGCCTGA TCGCGGCGGA GGCCCGCAAG AACGCGCCGG	840
CTCGTGGTGC CCGCAGGGCC CGCAAGTGAG GAGGCGATTA CACGCATGCG TGTTTGTGGC	900
TCTGAAGCGA CTTGGCGGGT CGGCTGTGAG GGTTCGAGAG GAGGTGTGTG ATGCGTGTGA	960
AGTCCTTCTC CGTTCTCAGC TCTCTCTGTG CTGTAGCTGT GCCTTTCCCC AGATCGCTTT	1020
ACCGCATTTC CATACTCTG TGTAGTCGCA TGTGCGTGTT TCTGTCTCTC GGTGGGTCTC	1080
CCTCTCCCTC CCTTTCTGCC TCTCTCTTTG AGTGGGTGTG CATGCGTCGC GCGCGACGGG	1140
CTCCGCTTNA GTGATTCTCT CGTGTTTTAN GGCTGTTTTY TTTCTYAGTT NAGCGTTTTY	1200
GTTTCATGATT TCCTCAGACC CAAAAAAAAA AAAAAAAAAA	1238

(2) INFORMATION FOR SEQ ID NO:76:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 712 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single

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(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

CTGACGGAGT TCCAGACGAA CCTTGTGCCG TACCCGCGCA TCCACTTCGT GCTGACAAGC	60
TACGCTCCGG TGGTGTCTGC CGAGAAGGCG TACCACGAGC AGCTNTCCGT CGCGGACATC	120
ACGAACTCGG TTTTGTAGCC TGCTGGCATG CTNACAAAGT GCGATCCTCG CCACGGCAAG	180
TACATGTCGT GCTGCCTCAT GTACCGCGGT GATGTCGTGC CGAAGGATGT CAACGCCGCG	240
ATTGCGACGA TCAAGACGAA GCGCACAATT CAGTTCGTGG ACTGGTGCCC GACCGGCTTC	300
AAGTGCGGCA TCAACTACCA GCCGCCGACC GTTGTGCCCC GCGGTGACCT CGCGAAGGTG	360
CAGCGCGCCG TGTGCATGAT TGCCAACTCG ACCGCGATCG CTGAGGTGTT TGCCCCGATC	420
GACCACAAGT TCGACCTGAT GTACAGCAAG CGCGCGTTTG TGCACTGGTA CGTGGGTGAG	480
GGCATGGAGG AGGGCGAGTT CTCCGAGGCG CGCGAGGATC TCGCTGCGCT GGAGAAGGAC	540
TACGAGGAGG TTGGCGCCGA GTCCGCCGAC GACATGGGCG AGGAGGACGT CGAGGAGTAC	600
TAAGGTAGAC TCGTGCCGCG CGCTGATGAT GTAGGTGCAC GCGTGCGTGT GCTGCAGCGG	660
AGCCGCCGCC ACCGCGACTG TGTGTGTGTG CGCGCGTGAC GACCGGCTCG AG	712

(2) INFORMATION FOR SEQ ID NO:77:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1086 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

CAAGAAGTGG ATCAAGCAGG AGACGAACGC CGATGGCGAG CGCGTGCGCC GCGCGTTCTG	60
CCAGTTCTGC CTAGACCCCA TCTACCAGAT CTTGACGCT GTGATGAACG AGAAGAAGGA	120
CAAGGTGGAC AAGATGCTCA AGTCGCTGCA CGTGACGCTN ACGGCTGAGG AGCGCGAGCA	180
GGTGCCGAAN AAGCTTCTGA AGACGGTGAT GATGAANTTC CTGCCGGCTG CTGAGACGCT	240
GCTACAGATG ATCGTGGCGC ACCTGCCGTC GCCCAAGAAG GCGCAGGCGT ACCGTGCGGA	300

GATGCTGTAC TCTGGCGAGG CGTCGCCGGA GGACAAGTAC TTCATGGGTA TCAAGAACTG	360
CGACCCCGCT GCGCCGCTCA TGCTGTACAT CAGCAAGATG GTGCCGACGG CCGACCGCGG	420
CCGCTTCTTC GCCTTTGGCC GCATCTTCTC CGGTAAGGTG CGCAGCGGCC AGAAGGTGCG	480
CATCATGGGT AACAACTACG TCTACGGCAA GAAGCAGGAC CTGTACGAGG ACAAGCCTGT	540
GCAGCGCTCC GTGCTGATGA TGGGCCGCTA CCAGGAGGCC GTGGAGGACA TGCCGTGCGG	600
TAACGTGGTG GGCCTTGTGG GCGTGGACAA GTACATCGTG AAGTCCGCGA CGATCACGGA	660
CGATGGCGAG AGCCCGCACC CGCTGCGCGA CATGAAGTAC TCTGTGTCGC CCGTCGTGCG	720
TGTGGCCGTG GAGGCGAAGA ACCCGTCCGA CCTGCCGAAG CTTGTGGAGG GCCTGAAGCG	780
CCTTGCCAAG TCCGACCCGC TGGTGGTGTG CAGCATTGAG GAGTCTGGCG AGCACATTGT	840
TGCCGGCGCT GGCGAGCTTC ACCTTGAGAT TTGCCTGAAG GATCTCCAGG AGGACTTCAT	900
GAACGGCGCG CCGCTNAAGA TCTCCGAGCC GGTGGTGTG TTCCGCGAGA CGGTGACGGA	960
TGTGTCGTG CAGCAGTGCC TGTCGAAGTC TGCGAACAAG CACAACCGTC TCTTCTGCCG	1020
CGGTGCGCCG CTNACAGAGG ANCTGGCGCT GGCGATNGAN GAAGGCACCG CTGGTCCCGA	1080
NGCGGA	1086

(2) INFORMATION FOR SEQ ID NO:78:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 447 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

CGCATCAACG TCTACTTCGA TNAGTCGACG GGAGGCCGCT ACGTGCCGCG CGCCGTGCTG	60
ATGGACCTCG AGCCCGGCAC TATGGACTCC GTTCGCGCCG GCCCGTACGG CCAGCTGTTC	120
CGCCCGGACA ACTTCATCTT TGGTCAGTCC GGCGCTGGCA ACAACTGGGC CAAGGGCCAC	180
TAACTGAGG GCGCGGAGCT GATCGACTCC GTGCTTGATG TGTGCCGCAA GGAGGCGGAG	240
AGCTGCGACT GCCTGCAGGG CTTCCAGCTG TCTCACTCCC TCGGCGGCGG CACGGGCTCC	300
GGCATGGGCA CGCTGCTCAT TTCCAANCTG CGCGANGAGT ACCCGGACCG GATCATGATG	360

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ACCTTCTCCG TCATCCCGTC CCCCCGCGTG TCGGATACCG TTGTGGANCC GTACAACACG 420

ACCCTCTCTG TGCACCAGCT CGTGGAA 447

(2) INFORMATION FOR SEQ ID NO:79:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 375 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

GTAACCCGCT GGTGTACGCA TATGTAGACA CAGACGGGCA GCACGAGACG ACGTTCCTCG 60
 CGATCCCTGT GGTGCTTGGC ATGAATGGAA TCGAGAAGCG CCTGCCGATT GGTCCGCTGC 120
 ACTCGACGGA GGAAACGCTG CTGAAGGCGG CACTGCCGGT GATCAAGAAG AATATCGTGA 180
 AGGGCAGCGA GTTCGCGCGC TCACACCTGT AGCACCTCAG CTTTTTTTTT TTGCGTTAAA 240
 CGGGCGTGGG AAGCACCTCG ATACTTCGCT TCGCGCTGAC GGACCCGCAC GACATCGTTC 300
 GTCATCCCCC TCCCCCTCTT CGGCCCTATA CGCATGAAGG AGTGGGAATTA TGCAACAGCA 360
 TGTNNATATC AAGTG 375

(2) INFORMATION FOR SEQ ID NO:80:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 107 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

Met Ala Ser Ser Arg Lys Ala Ser Asn Pro His Lys Ser His Arg Lys
 1 5 10 15
 Pro Lys Arg Ser Trp Asn Val Tyr Val Gly Arg Ser Leu Lys Ala Ile
 20 25 30
 Asn Ala Gln Met Ser Met Ser His Arg Thr Met Lys Ile Val Asn Ser
 35 40 45

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Tyr Val Asn Asp Val Met Glu Arg Ile Cys Thr Glu Ala Ala Ser Ile
 50 55 60
 Val Arg Ala Asn Lys Lys Arg Thr Leu Gly Ala Arg Glu Val Gln Thr
 65 70 75 80
 Ala Val Arg Ile Val Leu Pro Ala Glu Leu Ala Lys His Ala Met Ala
 85 90 95
 Glu Gly Thr Lys Ala Val Ser Ser Ala Ser Arg
 100 105

(2) INFORMATION FOR SEQ ID NO:81:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 381 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

Asp Glu Glu Glu Glu Asp Thr Thr Ile Asn Asn Ser Asp Val Val Val
 1 5 10 15
 Arg Tyr Lys Lys Ala Ala Thr Trp Cys Asn Glu Thr Leu Arg Val Leu
 20 25 30
 Ile Asp Ala Thr Lys Pro Gly Ala Lys Val Cys Asp Leu Cys Arg Leu
 35 40 45
 Gly Asp Asp Thr Ile Thr Ala Xaa Val Lys Thr Met Phe Lys Gly Thr
 50 55 60
 Glu Lys Gly Ile Ala Phe Pro Thr Cys Ile Ser Val Asn Asn Cys Val
 65 70 75 80
 Cys His Asn Ser Pro Gly Val Ser Asp Glu Thr Thr Gln Gln Glu Ile
 85 90 95
 Ala Met Gly Asp Val Val His Tyr Asp Leu Gly Ile His Val Asp Gly
 100 105 110
 Tyr Cys Ala Val Val Ala His Thr Ile Gln Val Thr Glu Asp Asn Glu
 115 120 125
 Leu Gly Lys Asp Glu Lys Ala Ala Arg Val Ile Thr Ala Ala Tyr Asn
 130 135 140
 Ile Leu Asn Thr Ala Leu Arg Gln Met Arg Pro Gly Thr Thr Ile Tyr
 145 150 155 160

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Gln Val Thr Asp Val Val Glu Lys Ala Ala Glu His Tyr Lys Val Thr
 165 170 175
 Pro Val Asp Gly Val Leu Ser His Met Met Lys Arg Tyr Ile Ile Asp
 180 185 190
 Xaa Tyr Arg Cys Ile Pro Gln Arg Arg Val Ala Glu His Met Val His
 195 200 205
 Asp Tyr Asp Leu Glu Lys Ala Gln Val Trp Thr Leu Asp Ile Val Met
 210 215 220
 Thr Ser Gly Lys Gly Lys Leu Lys Glu Arg Asp Ala Arg Pro Cys Val
 225 230 235 240
 Phe Lys Val Ala Leu Asp Ser Asn Tyr Ser Val Lys Met Glu Ser Ala
 245 250 255
 Lys Glu Val Gln Lys Glu Ile Asp Ser Xaa Tyr Ala Thr Phe Pro Phe
 260 265 270
 Ala Ile Arg Asn Leu Glu Ala Lys Lys Ala Arg Leu Gly Leu Asn Glu
 275 280 285
 Met Ala Lys His Gly Ala Val Ile Pro Tyr Pro Ile Leu Phe Glu Lys
 290 295 300
 Glu Gly Glu Val Val Ala His Phe Lys Ile Thr Val Leu Ile Ser Asn
 305 310 315 320
 Lys Lys Ile Glu Pro Ile Thr Gly Leu Lys Pro Gln Lys Ala Pro Ala
 325 330 335
 Leu Glu Pro Tyr Thr Asp Glu Met Leu Leu Ala Thr Asn Lys Leu Phe
 340 345 350
 Ala Val Ala Arg Glu Glu Gly Gly Glu Val Asp Gly Arg Gly Ile Arg
 355 360 365
 Asp Ala Val Leu Arg Ala Phe Val Gly Val Arg Leu Leu
 370 375 380

(2) INFORMATION FOR SEQ ID NO:82:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 191 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

```

Met Ser Ile Ile Lys Glu Asp Asp Ala Val Gly Cys Tyr Met Thr Val
1           5           10           15

Thr Leu Val Asp Asp Thr Lys Val Glu Gly Thr Ile Phe Thr Tyr Asn
          20           25           30

Ser Lys Glu Gly Ile Ile Val Leu Leu Ser Leu Arg Asp Asp Gln Thr
          35           40           45

Asn Met Lys Leu Ile Arg Thr Pro Tyr Ile Lys Asp Phe Ser Leu Ser
50           55           60

His Ala Glu Glu Gly Ala His Leu Pro Pro Ala Leu Asp Ser Phe Asn
65           70           75           80

Glu Leu Pro Ser Met His Ala Gly Arg Asp Lys Ser Ile Phe Lys His
          85           90           95

Ala Ser Thr Gln Leu Lys Asn Ala Glu Ala Asn Arg Glu Lys His Phe
          100          105          110

Asn Ser Val Thr Thr Asp Thr Pro Ile Ala Thr Leu Asp Ala Tyr Leu
          115          120          125

Lys Leu Leu Arg Leu Tyr Pro Leu Ile Glu Trp Asn Ser Asp Glu Gly
          130          135          140

Val Ile Gln Val Ser Asp Thr Val Ile Val Val Gly Asp Pro Asp Trp
          145          150          155          160

Arg Thr Pro Lys Ala Met Leu Val Asp Gly Ala Pro Glu Lys Asp Arg
          165          170          175

Pro Leu Val Asp Arg Leu Gln Val Ala Leu Gly Asn Gly Lys Lys
          180          185          190

```

(2) INFORMATION FOR SEQ ID NO:83:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 273 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

```

Met Ala Lys Lys His Leu Lys Arg Leu Tyr Ala Pro Lys Asp Trp Met
1           5           10           15

```

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Leu Ser Lys Leu Thr Gly Val Phe Ala Pro Arg Pro Arg Pro Gly Pro
 20 25 30
 His Lys Leu Arg Glu Cys Leu Pro Leu Leu Val Ile Ile Arg Asn Arg
 35 40 45
 Leu Lys Tyr Ala Leu Asn Ala Arg Glu Gly Glu Met Ile Leu Arg Gln
 50 55 60
 Gly Leu Val His Val Asp Asn His Pro Arg Arg Asp Gly Lys Tyr Pro
 65 70 75 80
 Ala Gly Phe Met Asp Val Val Glu Ile Pro Lys Thr Gly Asp Arg Phe
 85 90 95
 Arg Leu Met Tyr Asp Val Lys Gly Arg Phe Ala Leu Val Asn Leu Ser
 100 105 110
 Glu Ala Glu Ala Gln Ile Lys Leu Met Lys Val Val Asn Leu Tyr Thr
 115 120 125
 Ala Thr Gly Arg Val Pro Val Ala Val Thr His Asp Gly His Arg Ile
 130 135 140
 Arg Tyr Pro Asp Pro His Thr Ser Ile Gly Asp Thr Ile Val Tyr Asn
 145 150 155 160
 Val Lys Glu Lys Lys Cys Val Asp Leu Ile Lys Asn Arg Gln Gly Lys
 165 170 175
 Ala Val Ile Val Thr Gly Gly Ala Asn Arg Gly Arg Ile Gly Glu Ile
 180 185 190
 Val Lys Val Glu Cys His Pro Gly Ala Phe Asn Ile Ala His Leu Lys
 195 200 205
 Asp Ala Ser Gly Ala Glu Phe Ala Thr Arg Ala Ala Asn Ile Phe Val
 210 215 220
 Ile Gly Lys Asp Leu Asn Asn Leu Gln Val Thr Val Pro Lys Gln Gln
 225 230 235 240
 Gly Leu Arg Met Asn Val Ile Gln Glu Arg Glu Glu Arg Leu Ile Ala
 245 250 255
 Ala Glu Ala Arg Lys Asn Ala Pro Ala Arg Gly Ala Arg Arg Ala Arg
 260 265 270
 Lys

(2) INFORMATION FOR SEQ ID NO:84:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 200 amino acids

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- (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

```

Leu Thr Glu Phe Gln Thr Asn Leu Val Pro Tyr Pro Arg Ile His Phe
1           5           10           15

Val Leu Thr Ser Tyr Ala Pro Val Val Ser Ala Glu Lys Ala Tyr His
          20           25           30

Glu Gln Leu Ser Val Ala Asp Ile Thr Asn Ser Val Phe Glu Pro Ala
          35           40           45

Gly Met Leu Thr Lys Cys Asp Pro Arg His Gly Lys Tyr Met Ser Cys
          50           55           60

Cys Leu Met Tyr Arg Gly Asp Val Val Pro Lys Asp Val Asn Ala Ala
          65           70           75           80

Ile Ala Thr Ile Lys Thr Lys Arg Thr Ile Gln Phe Val Asp Trp Cys
          85           90           95

Pro Thr Gly Phe Lys Cys Gly Ile Asn Tyr Gln Pro Pro Thr Val Val
          100          105          110

Pro Gly Gly Asp Leu Ala Lys Val Gln Arg Ala Val Cys Met Ile Ala
          115          120          125

Asn Ser Thr Ala Ile Ala Glu Val Phe Ala Arg Ile Asp His Lys Phe
          130          135          140

Asp Leu Met Tyr Ser Lys Arg Ala Phe Val His Trp Tyr Val Gly Glu
          145          150          155          160

Gly Met Glu Glu Gly Glu Phe Ser Glu Ala Arg Glu Asp Leu Ala Ala
          165          170          175

Leu Glu Lys Asp Tyr Glu Glu Val Gly Ala Glu Ser Ala Asp Asp Met
          180          185          190

Gly Glu Glu Asp Val Glu Glu Tyr
          195          200

```

(2) INFORMATION FOR SEQ ID NO:85:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 361 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS:

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(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

Lys	Lys	Trp	Ile	Lys	Gln	Glu	Thr	Asn	Ala	Asp	Gly	Glu	Arg	Val	Arg	1	5	10	15
Arg	Ala	Phe	Cys	Gln	Phe	Cys	Leu	Asp	Pro	Ile	Tyr	Gln	Ile	Phe	Asp	20	25	30	
Ala	Val	Met	Asn	Glu	Lys	Lys	Asp	Lys	Val	Asp	Lys	Met	Leu	Lys	Ser	35	40	45	
Leu	His	Val	Thr	Leu	Thr	Ala	Glu	Glu	Arg	Glu	Gln	Val	Pro	Xaa	Lys	50	55	60	
Leu	Leu	Lys	Thr	Val	Met	Met	Xaa	Phe	Leu	Pro	Ala	Ala	Glu	Thr	Leu	65	70	75	80
Leu	Gln	Met	Ile	Val	Ala	His	Leu	Pro	Ser	Pro	Lys	Lys	Ala	Gln	Ala	85	90	95	
Tyr	Arg	Ala	Glu	Met	Leu	Tyr	Ser	Gly	Glu	Ala	Ser	Pro	Glu	Asp	Lys	100	105	110	
Tyr	Phe	Met	Gly	Ile	Lys	Asn	Cys	Asp	Pro	Ala	Ala	Pro	Leu	Met	Leu	115	120	125	
Tyr	Ile	Ser	Lys	Met	Val	Pro	Thr	Ala	Asp	Arg	Gly	Arg	Phe	Phe	Ala	130	135	140	
Phe	Gly	Arg	Ile	Phe	Ser	Gly	Lys	Val	Arg	Ser	Gly	Gln	Lys	Val	Arg	145	150	155	160
Ile	Met	Gly	Asn	Asn	Tyr	Val	Tyr	Gly	Lys	Lys	Gln	Asp	Leu	Tyr	Glu	165	170	175	
Asp	Lys	Pro	Val	Gln	Arg	Ser	Val	Leu	Met	Met	Gly	Arg	Tyr	Gln	Glu	180	185	190	
Ala	Val	Glu	Asp	Met	Pro	Cys	Gly	Asn	Val	Val	Gly	Leu	Val	Gly	Val	195	200	205	
Asp	Lys	Tyr	Ile	Val	Lys	Ser	Ala	Thr	Ile	Thr	Asp	Asp	Gly	Glu	Ser	210	215	220	
Pro	His	Pro	Leu	Arg	Asp	Met	Lys	Tyr	Ser	Val	Ser	Pro	Val	Val	Arg	225	230	235	240
Val	Ala	Val	Glu	Ala	Lys	Asn	Pro	Ser	Asp	Leu	Pro	Lys	Leu	Val	Glu	245	250	255	

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Gly Leu Lys Arg Leu Ala Lys Ser Asp Pro Leu Val Val Cys Ser Ile
 260 265 270
 Glu Glu Ser Gly Glu His Ile Val Ala Gly Ala Gly Glu Leu His Leu
 275 280 285
 Glu Ile Cys Leu Lys Asp Leu Gln Glu Asp Phe Met Asn Gly Ala Pro
 290 295 300
 Leu Lys Ile Ser Glu Pro Val Val Ser Phe Arg Glu Thr Val Thr Asp
 305 310 315 320
 Val Ser Ser Gln Gln Cys Leu Ser Lys Ser Ala Asn Lys His Asn Arg
 325 330 335
 Leu Phe Cys Arg Gly Ala Pro Leu Thr Glu Xaa Leu Ala Leu Ala Xaa
 340 345 350
 Xaa Glu Gly Thr Ala Gly Pro Xaa Ala
 355 360

(2) INFORMATION FOR SEQ ID NO:86:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 149 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

Arg Ile Asn Val Tyr Phe Asp Xaa Ser Thr Gly Gly Arg Tyr Val Pro
 1 5 10 15
 Arg Ala Val Leu Met Asp Leu Glu Pro Gly Thr Met Asp Ser Val Arg
 20 25 30
 Ala Gly Pro Tyr Gly Gln Leu Phe Arg Pro Asp Asn Phe Ile Phe Gly
 35 40 45
 Gln Ser Gly Ala Gly Asn Asn Trp Ala Lys Gly His Tyr Thr Glu Gly
 50 55 60
 Ala Glu Leu Ile Asp Ser Val Leu Asp Val Cys Arg Lys Glu Ala Glu
 65 70 75 80
 Ser Cys Asp Cys Leu Gln Gly Phe Gln Leu Ser His Ser Leu Gly Gly
 85 90 95
 Gly Thr Gly Ser Gly Met Gly Thr Leu Leu Ile Ser Xaa Leu Arg Xaa

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	100		105		110
Glu Tyr Pro Asp Arg Ile Met Met Thr Phe Ser Val Ile Pro Ser Pro					
115		120		125	
Arg Val Ser Asp Thr Val Val Xaa Pro Tyr Asn Thr Thr Leu Ser Val					
130		135		140	
His Gln Leu Val Glu					
145					

(2) INFORMATION FOR SEQ ID NO:87:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 69 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

Asn Pro Leu Val Tyr Ala Tyr Val Asp Thr Asp Gly Gln His Glu Thr			
1	5	10	15
Thr Phe Leu Ala Ile Pro Val Val Leu Gly Met Asn Gly Ile Glu Lys			
20	25	30	
Arg Leu Pro Ile Gly Pro Leu His Ser Thr Glu Glu Thr Leu Leu Lys			
35	40	45	
Ala Ala Leu Pro Val Ile Lys Lys Asn Ile Val Lys Gly Ser Glu Phe			
50	55	60	
Ala Arg Ser His Leu			
65			

CLAIMS

1. A polypeptide comprising an immunogenic portion of a *Leishmania* antigen having the amino acid sequence recited in SEQ ID NO:2, or a variant of said antigen that differs only in conservative substitutions and/or modifications.
2. The polypeptide of claim 1, comprising amino acids 1 - 564 of SEQ ID NO:2.
3. An isolated DNA molecule comprising a nucleotide sequence encoding a polypeptide according to claim 1.
4. The DNA molecule of claim 3 wherein the nucleotide sequence is selected from the group consisting of:
 - (a) nucleotides 421 through 2058 of SEQ ID NO:1; and
 - (b) DNA sequences that hybridize to a nucleotide sequence complementary to nucleotides 421 through 2058 of SEQ ID NO:1 under moderately stringent conditions.
5. A recombinant expression vector comprising the DNA molecule of claim 3.
6. A host cell transformed or transfected with the expression vector of claim 5.
7. A polypeptide comprising an immunogenic portion of a *Leishmania* antigen having the amino acid sequence recited in SEQ ID NO:4, or a variant of said antigen that differs only in conservative substitutions and/or modifications.

8. The polypeptide of claim 7, comprising amino acids 1-175 of SEQ ID NO:4.
9. An isolated DNA molecule comprising a nucleotide sequence encoding a polypeptide according to claim 7.
10. The DNA molecule of claim 9 wherein the nucleotide sequence is selected from the group consisting of:
 - (a) nucleotides 25 through 549 of SEQ ID NO:3; and
 - (b) DNA sequences that hybridize to a nucleotide sequence complementary to nucleotides 25 through 549 of SEQ ID NO:3 under moderately stringent conditions.
11. A recombinant expression vector comprising the DNA molecule of claim 9.
12. A host cell transformed or transfected with the expression vector of claim 11.
13. A polypeptide comprising an immunogenic portion of a *Leishmania* antigen or a variant of said antigen that differs only in conservative substitutions and/or modifications, wherein said antigen comprises an amino acid sequence selected from the group consisting of sequences recited in SEQ ID NO: 22, 24, 26, 36-38, 41, 49-53 and 82.
14. An antigenic epitope of a *Leishmania* antigen comprising an amino acid sequence recited in SEQ ID NO:43.
15. A polypeptide comprising at least two contiguous antigenic epitopes according to claim 14.

16. An isolated DNA molecule comprising a nucleotide sequence encoding a polypeptide according to claims 13 or 15.

17. The DNA molecule of claim 16 wherein the nucleotide sequence is selected from the group consisting of:

(a) sequences recited in SEQ ID NO: 21, 23, 25, 29-31, 34, 45-48 and 74; and

(b) DNA sequences that hybridize to a nucleotide sequence complementary to a sequence recited in SEQ ID NO: 21, 23, 25, 29-31, 34, 45-48 and 74 under moderately stringent conditions.

18. An expression vector comprising the DNA molecule of claim 16.

19. A host cell transformed or transfected with the expression vector of claim 18.

20. A polypeptide comprising an immunogenic portion of a *Leishmania* antigen or a variant of said antigen that differs only in conservative substitutions and/or modifications, wherein said antigen comprises an amino acid sequence provided in SEQ ID NO: 20.

21. An isolated DNA molecule comprising a nucleotide sequence encoding a polypeptide according to claim 20.

22. The DNA molecule of claim 21 wherein the nucleotide sequence is selected from the group consisting of:

(a) a sequence recited in SEQ ID NO: 19; and

(b) DNA sequences that hybridize to a nucleotide sequence complementary to the sequence of SEQ ID NO: 19 under moderately stringent conditions.

23. An expression vector comprising the DNA molecule of claim 21.
24. A host cell transformed or transfected with the expression vector of claim 23.
25. A pharmaceutical composition comprising a polypeptide according to any one of claims 1, 7, 13 and 15, and a physiologically acceptable carrier.
26. A pharmaceutical composition comprising a polypeptide according to claim 20 and a physiologically acceptable carrier.
27. A pharmaceutical composition comprising at least two different polypeptides selected from the group consisting of:
- (a) a polypeptide according to claim 1;
 - (b) a polypeptide according to claim 7;
 - (c) a polypeptide according to claim 13
 - (d) a polypeptide according to claim 15;
 - (e) a polypeptide according to claim 20;
 - (f) a polypeptide comprising an immunogenic portion of a *Leishmania* antigen having the amino acid sequence recited in SEQ ID NO:6, or a variant of said antigen that differs only in conservative substitutions and/or modifications;
 - (g) a polypeptide comprising an immunogenic portion of a *Leishmania* antigen having the amino acid sequence recited in SEQ ID NO:8, or a variant of said antigen that differs only in conservative substitutions and/or modifications; and
 - (h) a polypeptide comprising an immunogenic portion of a *Leishmania* antigen having the amino acid sequence recited in SEQ ID NO:10, or a variant of said antigen that differs only in conservative substitutions and/or modifications;
- and a physiologically acceptable carrier.

28. A pharmaceutical composition according to any one of claims 25, 26 and 27, further comprising soluble *Leishmania* antigens.

29. A pharmaceutical composition according to any one of claims 25, 26 and 27, further comprising a K39 *Leishmania* antigen.

30. A pharmaceutical composition comprising soluble *Leishmania* antigens and a physiologically acceptable carrier.

31. A vaccine comprising a polypeptide according to any one of claims 1, 7, 13 and 15, and a non-specific immune response enhancer.

32. A vaccine comprising a polypeptide according to claim 20 and a non-specific immune response enhancer.

33. A vaccine comprising at least two different polypeptides selected from the group consisting of:

- (a) a polypeptide according to claim 1;
- (b) a polypeptide according to claim 7;
- (c) a polypeptide according to claim 13;
- (d) a polypeptide according to claim 15
- (e) a polypeptide according to claim 21;
- (f) a polypeptide comprising an immunogenic portion of a *Leishmania* antigen having the amino acid sequence recited in SEQ ID NO:6, or a variant of said antigen that differs only in conservative substitutions and/or modifications;
- (g) a polypeptide comprising an immunogenic portion of a *Leishmania* antigen having the amino acid sequence recited in SEQ ID NO:8, or a variant of said antigen that differs only in conservative substitutions and/or modifications; and

(h) a polypeptide comprising an immunogenic portion of a *Leishmania* antigen having the amino acid sequence recited in SEQ ID NO:10, or a variant of said antigen that differs only in conservative substitutions and/or modifications;

and a non-specific immune response enhancer.

34. A vaccine comprising soluble *Leishmania* antigens and a non-specific immune response enhancer.

35. A vaccine according to any one of claims 31, 32, 33 and 34 wherein the non-specific immune response enhancer is an immunogenic portion of a *Leishmania* antigen having the amino acid sequence recited in SEQ ID NO:10, or a variant of said antigen that differs only in conservative substitutions and/or modifications.

36. A vaccine according to any one of claims 31, 32 and 33, further comprising soluble *Leishmania* antigens.

37. A vaccine according to claim 36 wherein the non-specific immune response enhancer is an immunogenic portion of a *Leishmania* antigen having the amino acid sequence recited in SEQ ID NO:10, or a variant of said antigen that differs only in conservative substitutions and/or modifications.

38. A vaccine according to any one of claims 31, 32, 33 and 34, further comprising a delivery vehicle.

39. The vaccine of claim 38 wherein the delivery vehicle is a biodegradable microsphere.

40. A vaccine comprising a DNA molecule according to any one of claims 3, 9 and 16.

41. A vaccine comprising a DNA molecule according to claim 21.
42. A pharmaceutical composition according to any one of claims 25, 26, 27, 29 and 30, for use in the manufacture of a medicament for inducing protective immunity against leishmaniasis in a patient.
43. The composition of claim 42 wherein the leishmaniasis is caused by a *Leishmania* species selected from the group consisting of *L. donovani*, *L. chagasi*, *L. infantum*, *L. major*, *L. amazonensis*, *L. braziliensis*, *L. panamensis*, *L. tropica* and *L. guyanensis*.
44. A vaccine according to any one of claims 31-34, 40 and 41, for use in a method for inducing protective immunity against leishmaniasis in a patient comprising administering.
45. The vaccine of claim 44 wherein the leishmaniasis is caused by a *Leishmania* species selected from the group consisting of *L. donovani*, *L. chagasi*, *L. infantum*, *L. major*, *L. amazonensis*, *L. braziliensis*, *L. panamensis*, *L. tropica* and *L. guyanensis*.
46. A pharmaceutical composition according to any one of claims 25, 26, 27, 29 and 30, for use in a method for detecting *Leishmania* infection in a patient.
47. A diagnostic kit comprising:
- (a) a pharmaceutical composition according to any one of claims 25, 26, 27, 29 and 30; and
 - (b) apparatus sufficient to contact dermal cells of a patient with the pharmaceutical composition.

48. A pharmaceutical composition according to any one of claims 25, 26, 27, 29 and 30, for use in the manufacture of a medicament for stimulating a cellular and/or humoral immune response in a patient.

49. The composition of claim 48 wherein said response is a Th1¹ immune response.

50. The composition of claim 48 wherein said response is IL-12 production.

51. A vaccine according to any of claims 31-34, 40 and 41, for use in the manufacture of a medicament for stimulating a cellular and/or humoral immune response in a patient.

52. The vaccine of claim 51 wherein said response is a Th1 response.

53. The method of claim 51 wherein said response is IL-12 production.

54. A pharmaceutical composition according to any one of claims 25, 26, 27, 29 and 30, for use in the manufacture of a medicament for treating a patient afflicted with a disease responsive to IL-12 stimulation.

55. A vaccine according to any one of claims 31-34, 40 and 41, for use in the manufacture of a medicament for treating a patient afflicted with a disease responsive to IL-12 stimulation.

56. A pharmaceutical composition comprising a polypeptide and a physiologically acceptable carrier, the polypeptide comprising an immunogenic portion of a *Leishmania* antigen or a variant of said antigen that differs only in conservative substitutions

and/or modifications, wherein said antigen comprises an amino acid sequence selected from the group consisting of SEQ ID NO:39, 42, 55, 61, 62, 80, 81, and 83-87.

57. A pharmaceutical composition according to claim 56 further comprising a K39 *Leishmania* antigen.

58. A vaccine comprising a polypeptide and a non-specific immune response enhancer, the polypeptide comprising an immunogenic portion of a *Leishmania* antigen or a variant of said antigen that differs only in conservative substitutions and/or modifications, wherein said antigen comprises an amino acid sequence selected from the group consisting of SEQ ID NO:39, 42, 55, 61, 62, 80, 81 and 83-87.

59. A pharmaceutical composition according to any one of claims 56 and 57, for use in the manufacture of a medicament for inducing protective immunity against leishmaniasis in a patient.

60. A vaccine according to claim 58, for use in the manufacture of a medicament for inducing protective immunity against leishmaniasis in a patient.

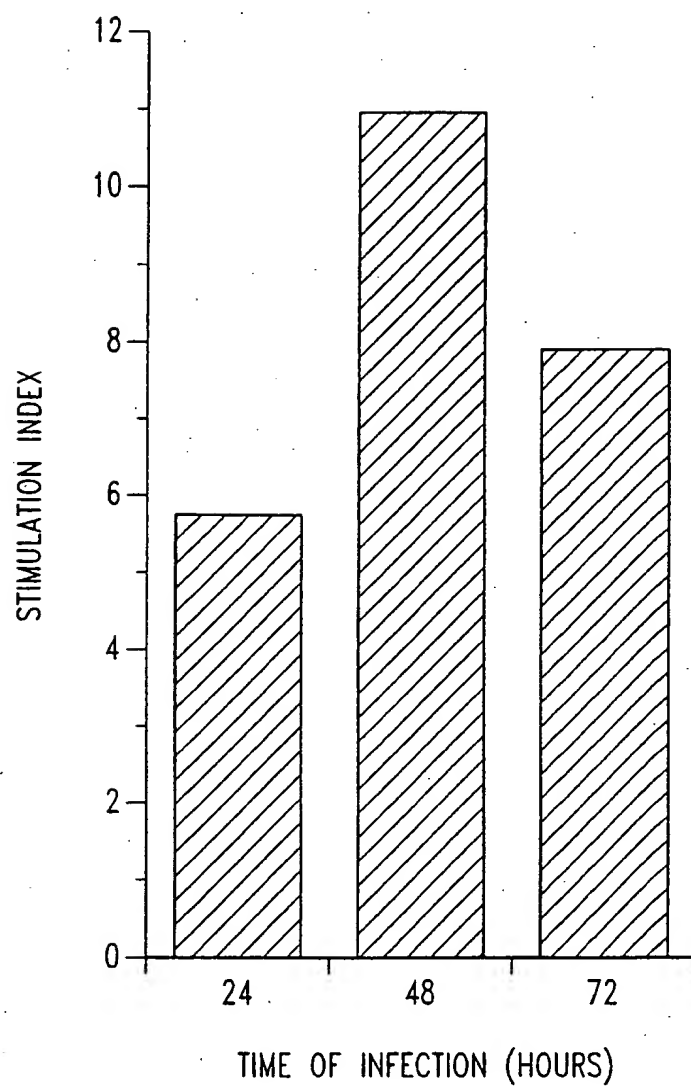
61. A pharmaceutical composition according to any one of claims 56 or 57, for use in a method for detecting *Leishmania* infection in a patient.

62. A pharmaceutical composition according to claim 56, and a composition comprising a K39 *Leishmania* antigen, for use in a method for detecting *Leishmania* infection in a patient.

63. A diagnostic kit comprising:

- (a) a pharmaceutical composition according to any one of claims 56 and 57; and
- (b) apparatus sufficient to contact dermal cells of a patient with the pharmaceutical composition.

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*Fig. 1*

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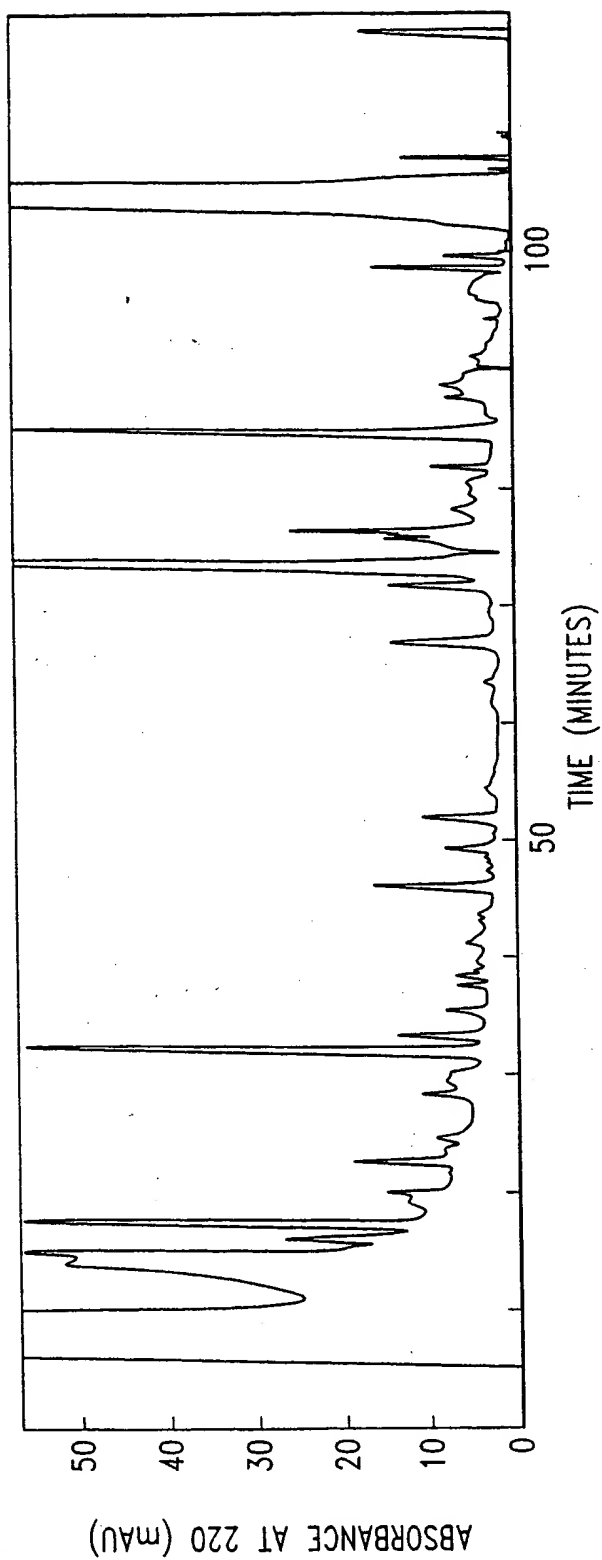


Fig. 2A

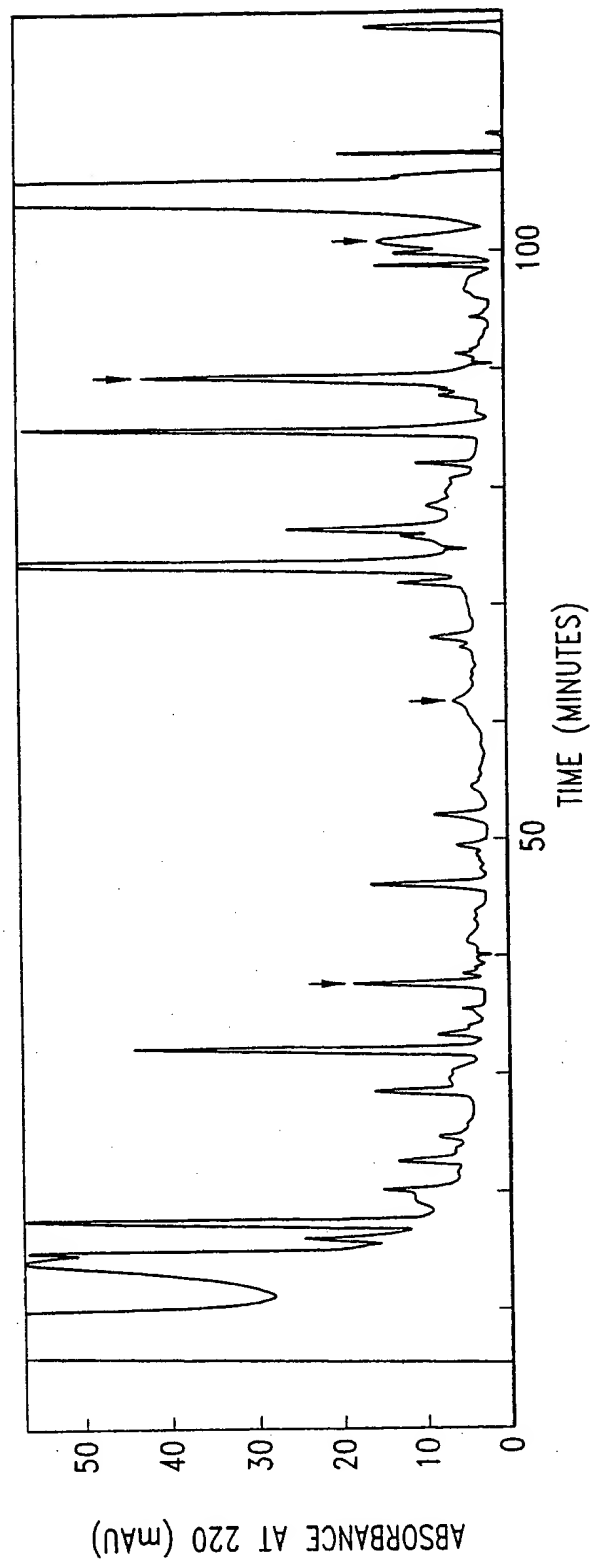


Fig. 2B

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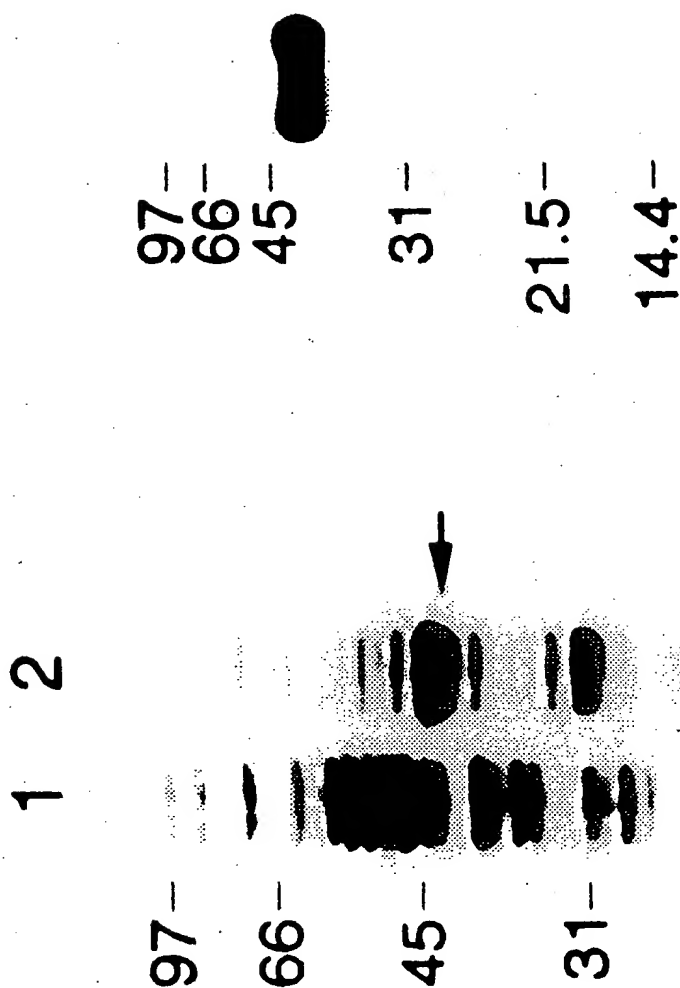
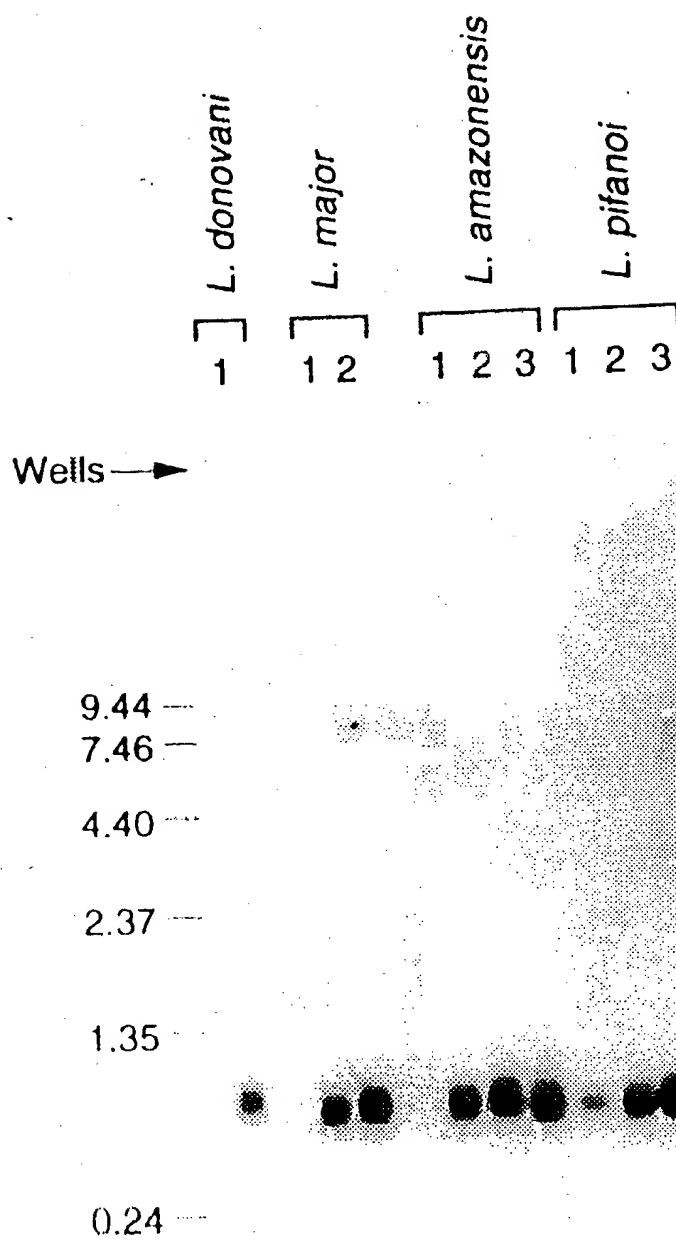


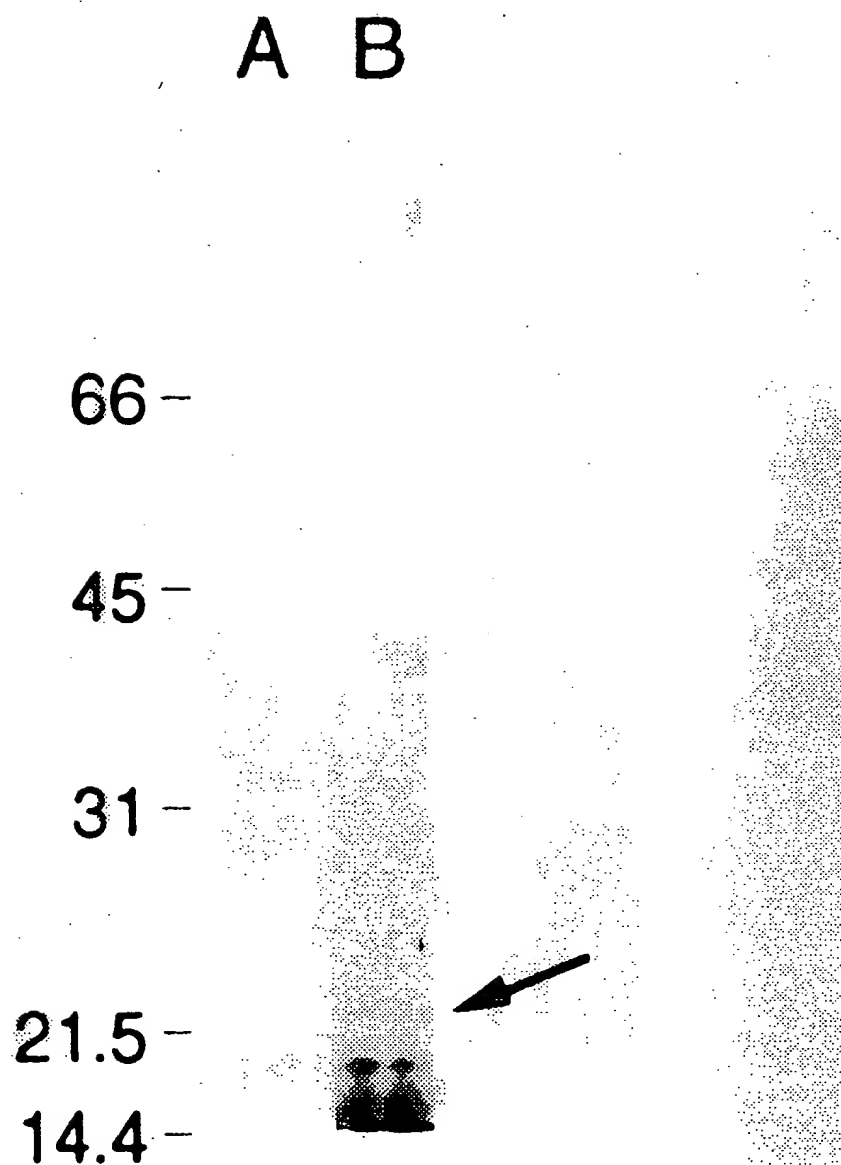
Fig. 3B

Fig. 3A

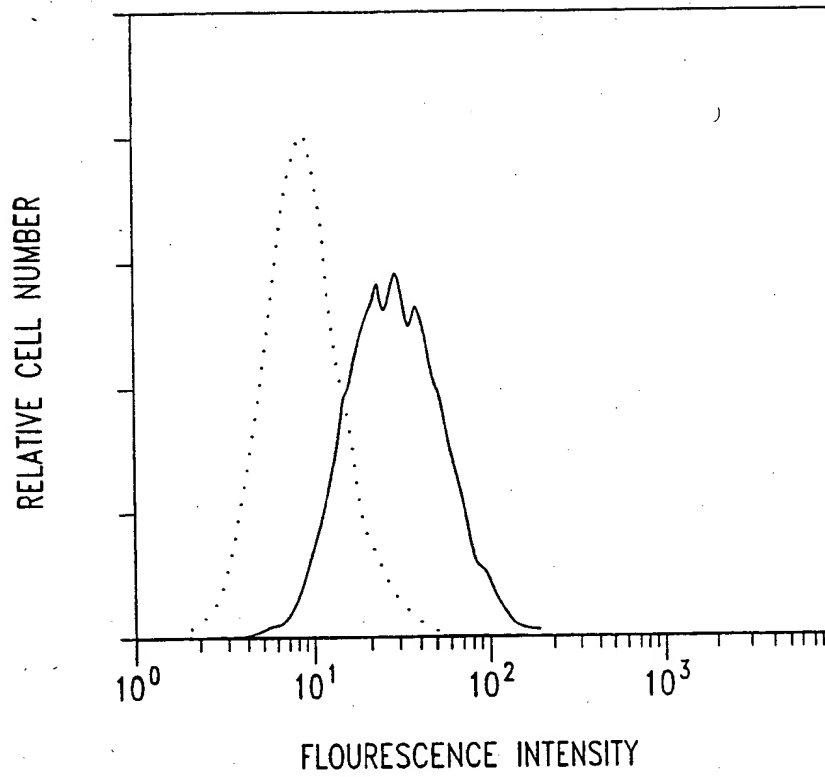
4/29

*Fig. 4*

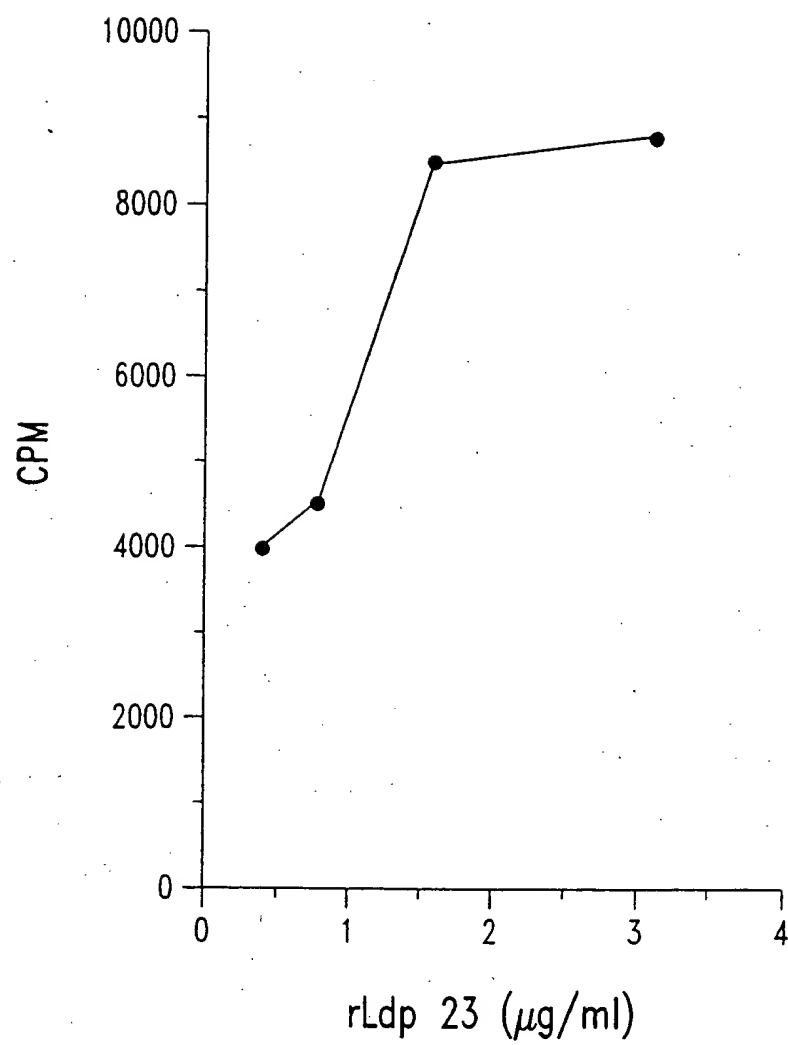
5/29

*Fig. 5*

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*Fig. 6*

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*Fig. 7*

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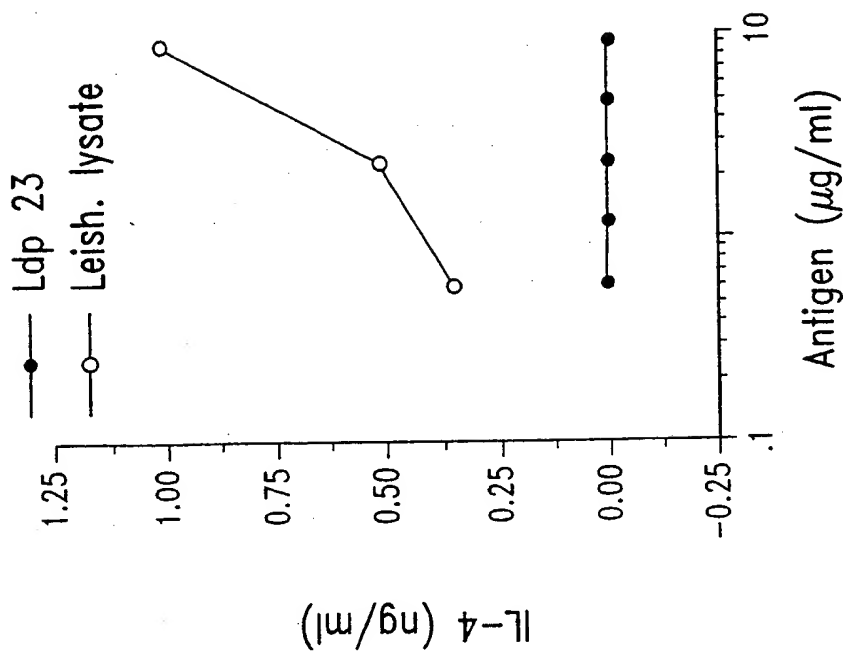


Fig. 8B

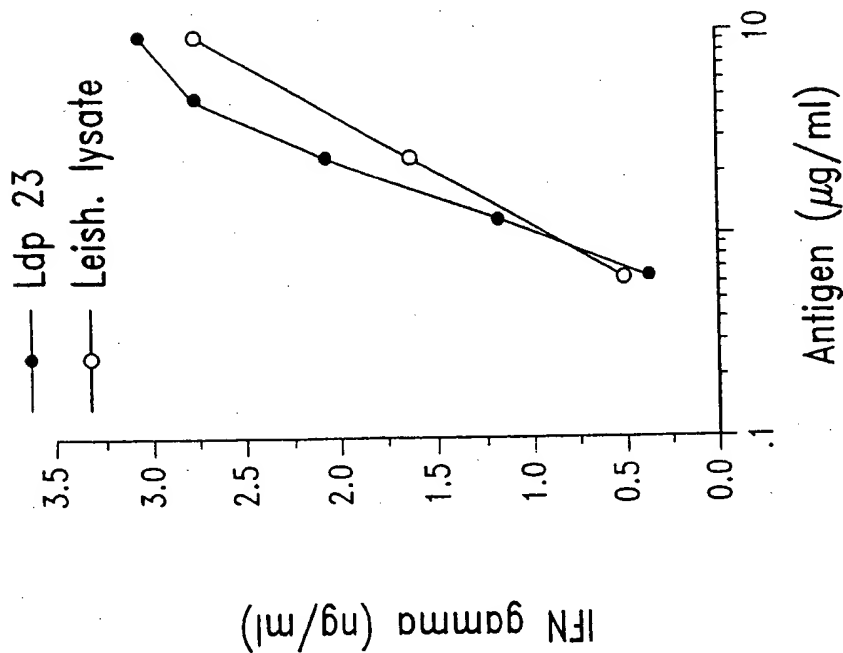


Fig. 8A

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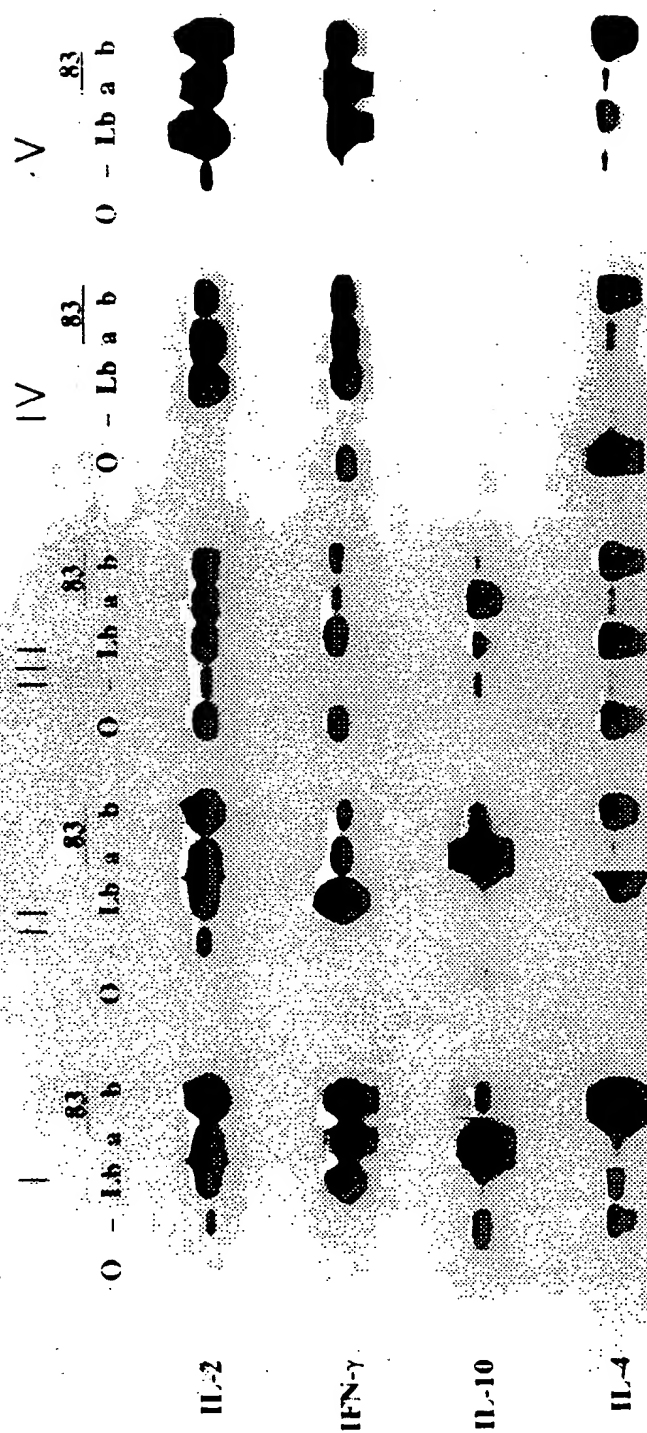


Fig. 9A

10/29

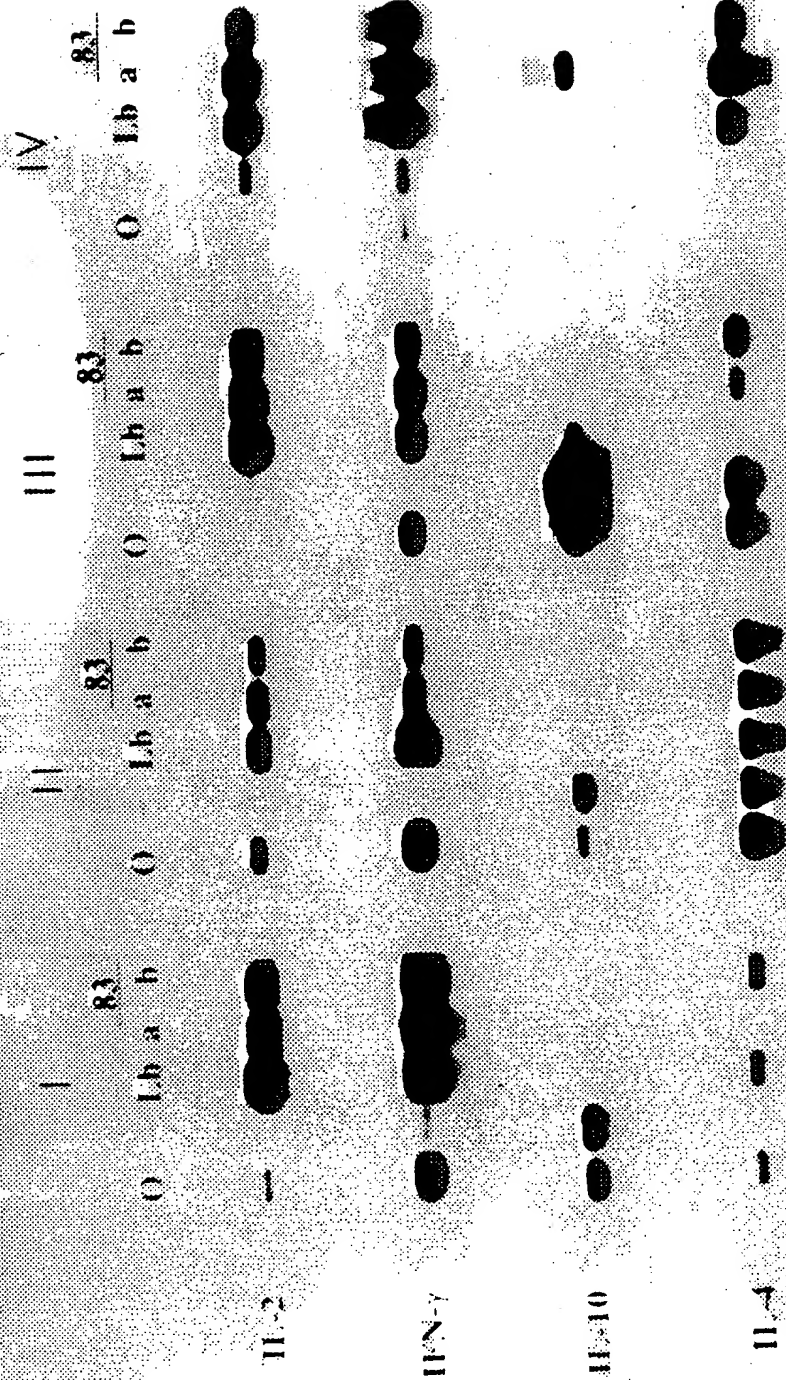
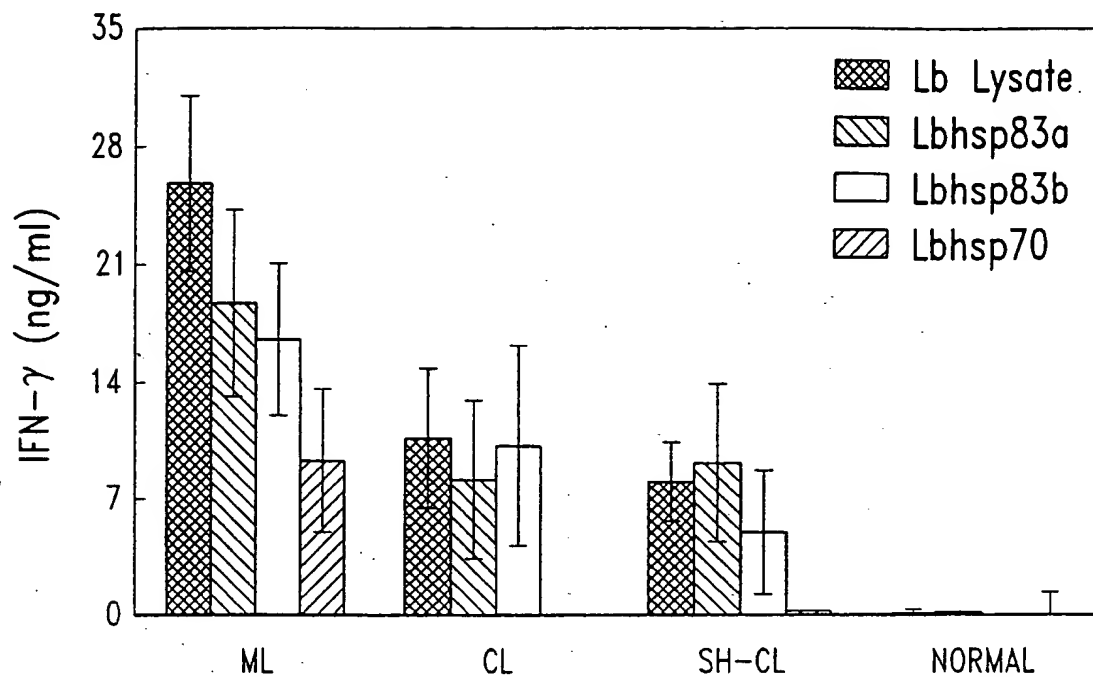
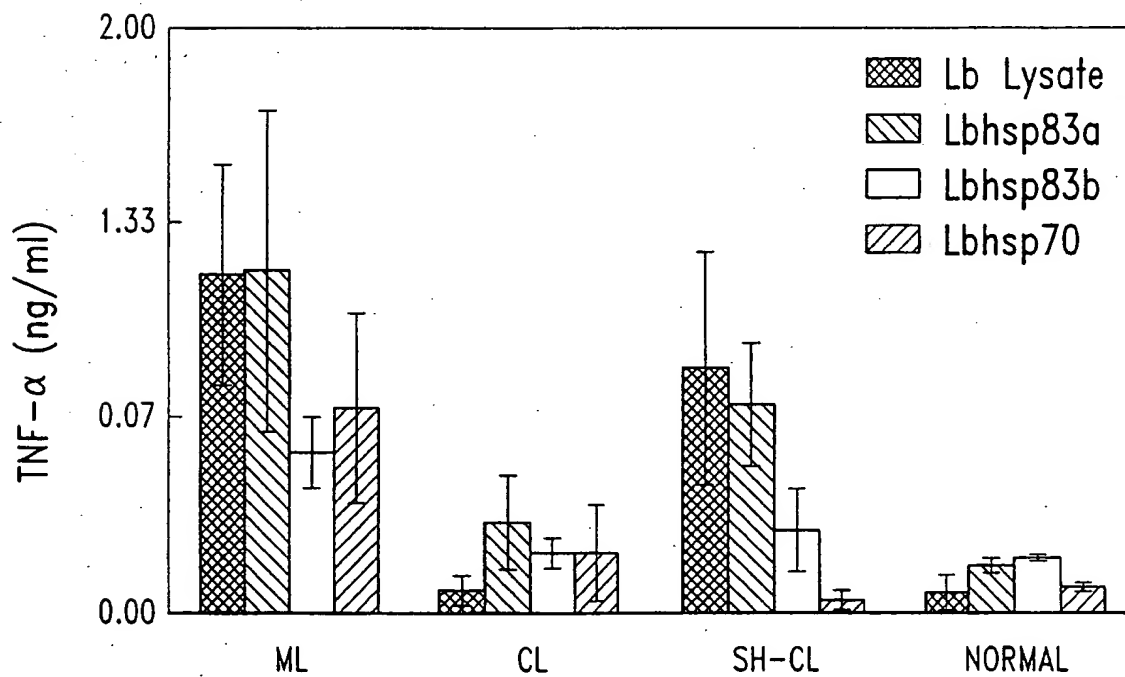
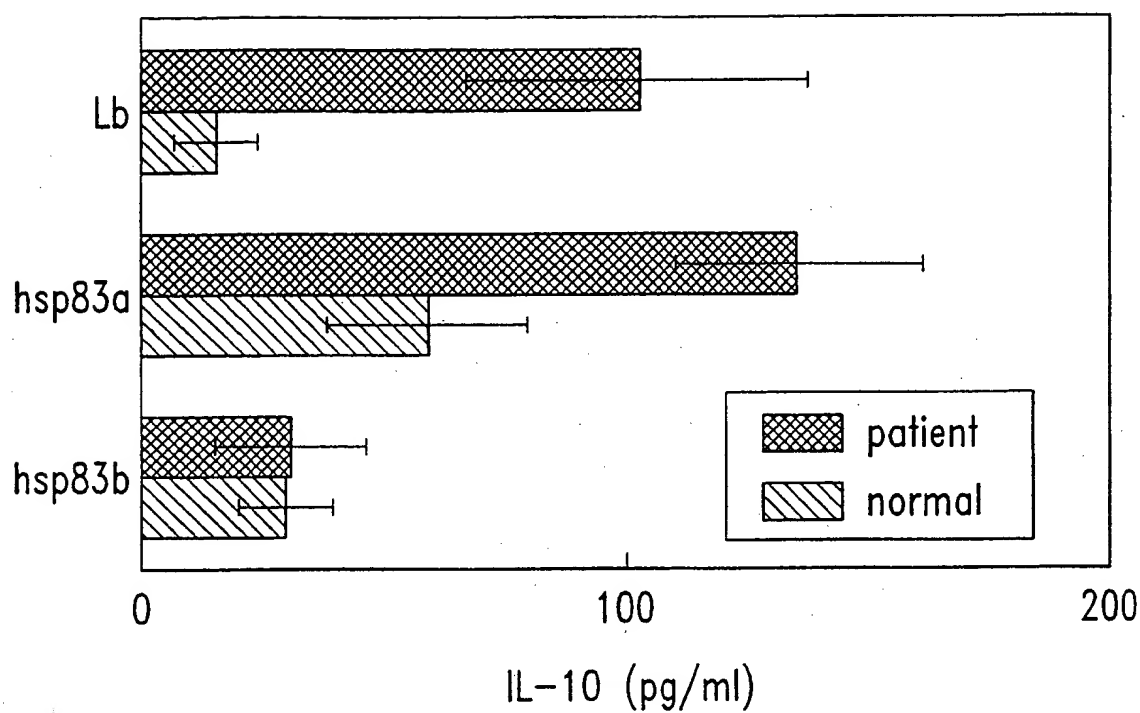
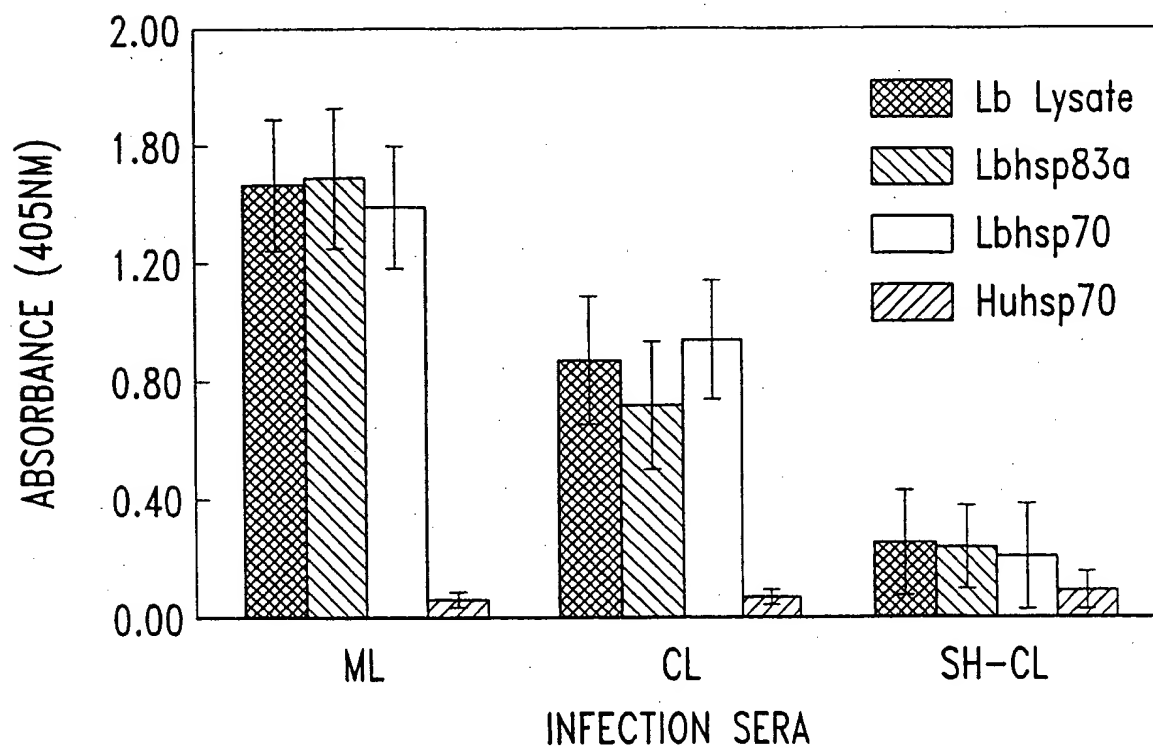


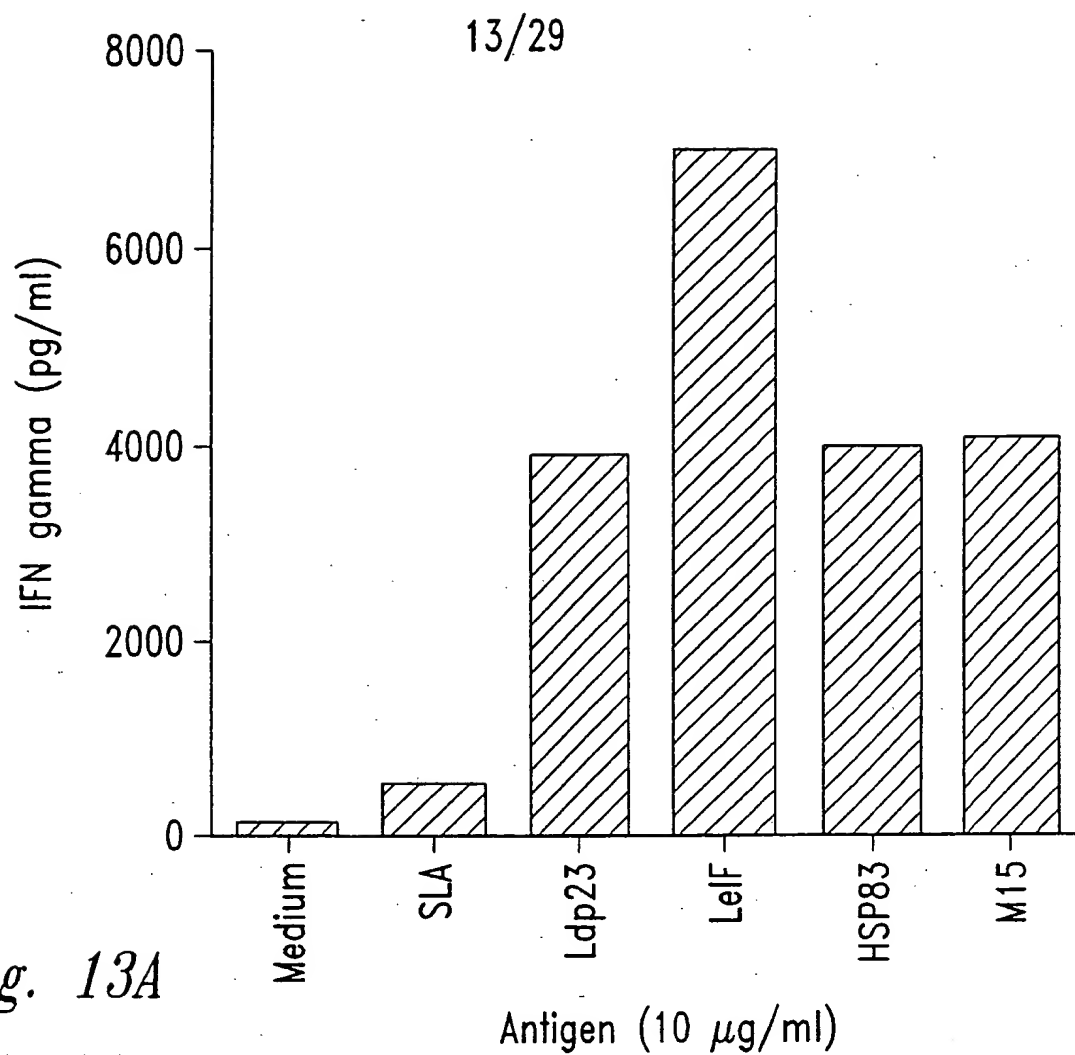
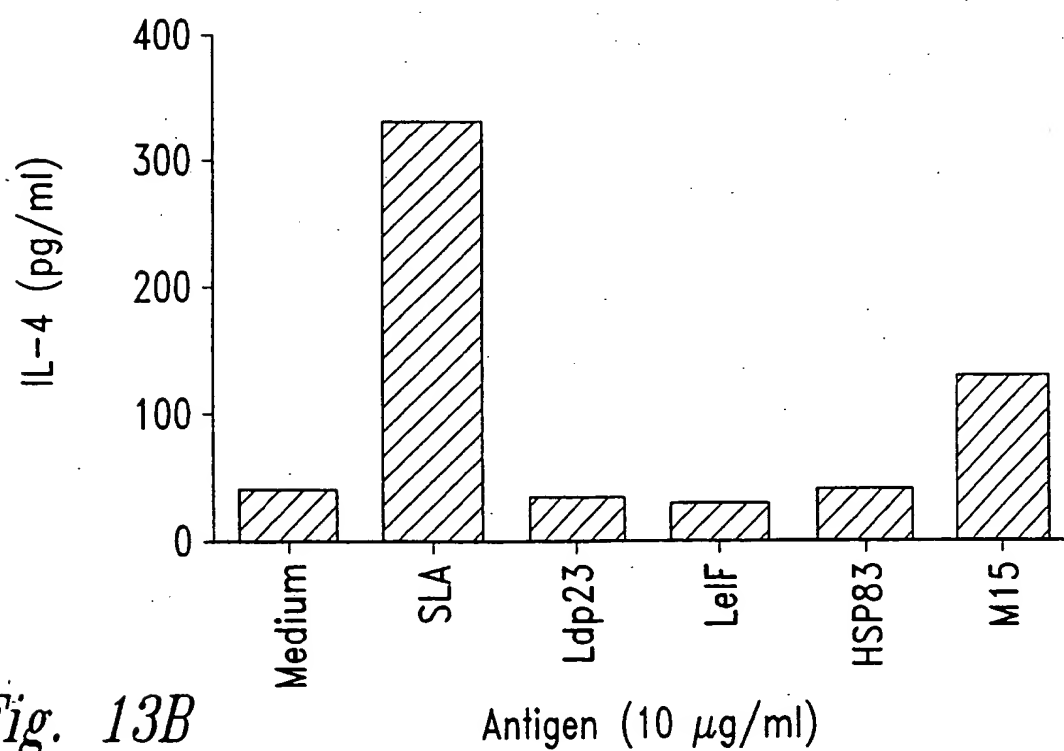
Fig. 9B

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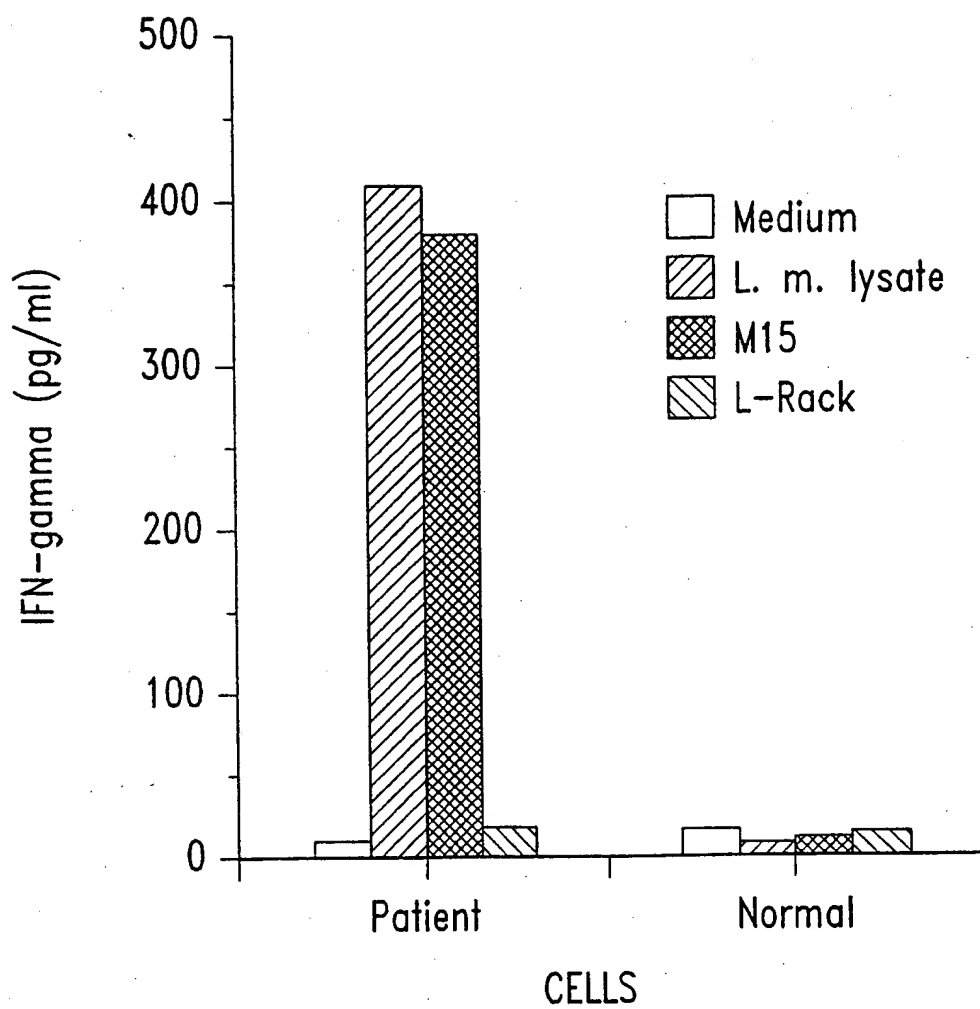
*Fig. 10A**Fig. 10B*

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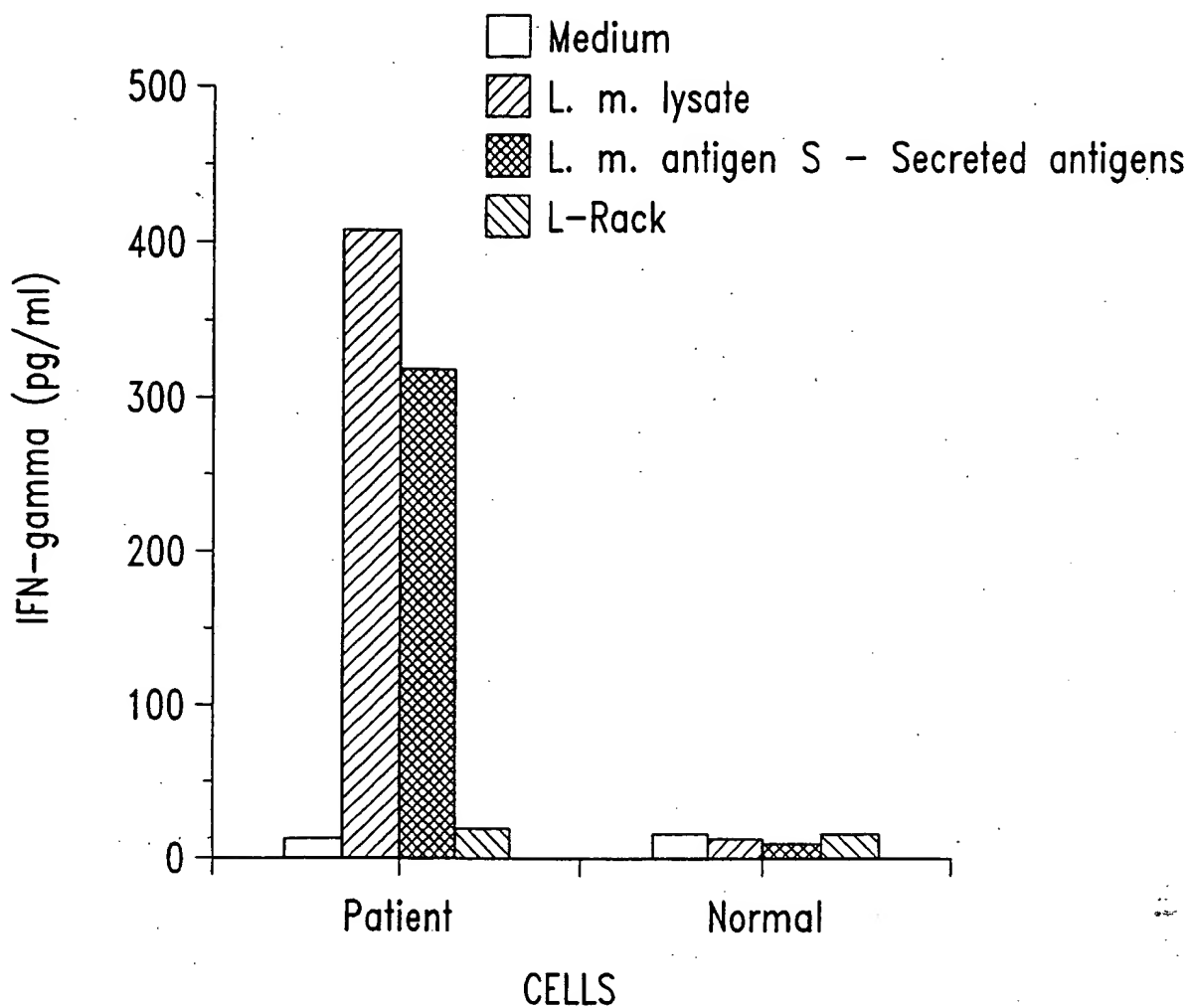
*Fig. 11**Fig. 12*

*Fig. 13A**Fig. 13B*

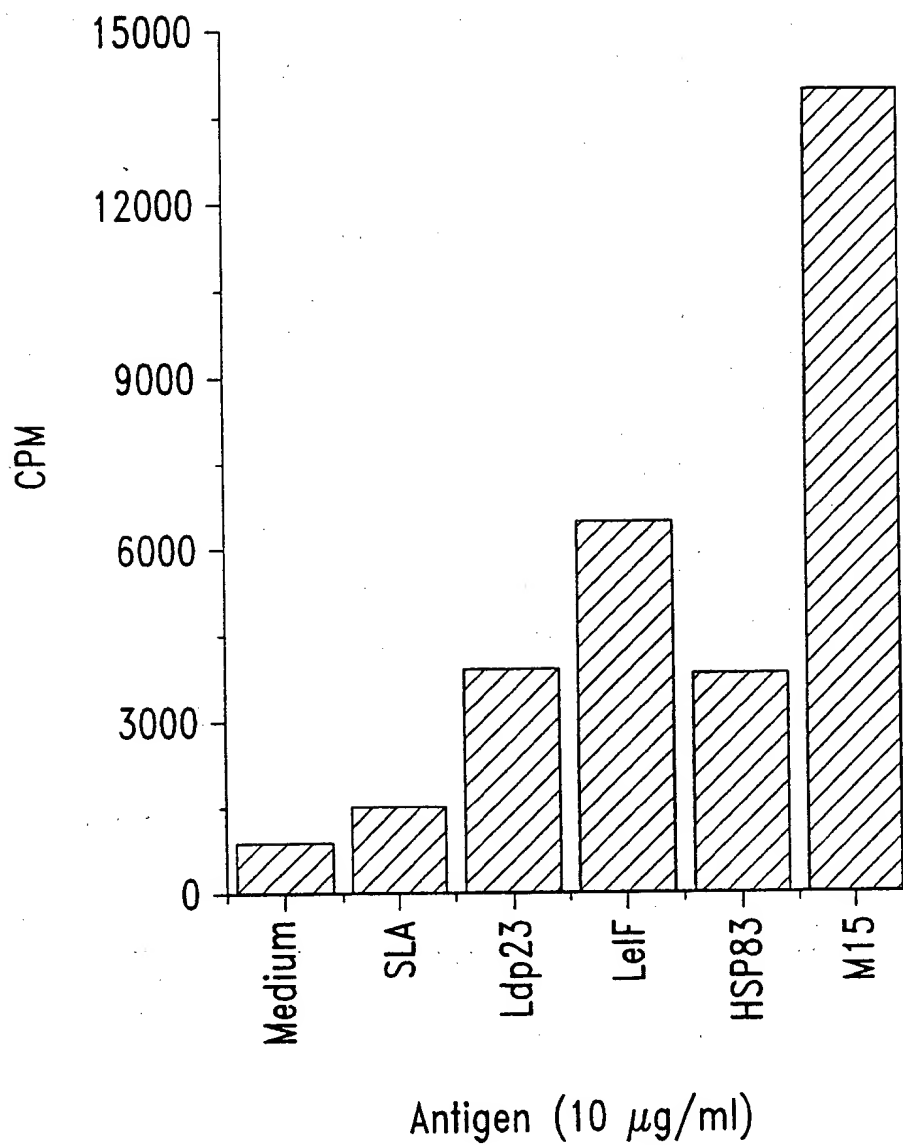
14/29

*Fig. 14*

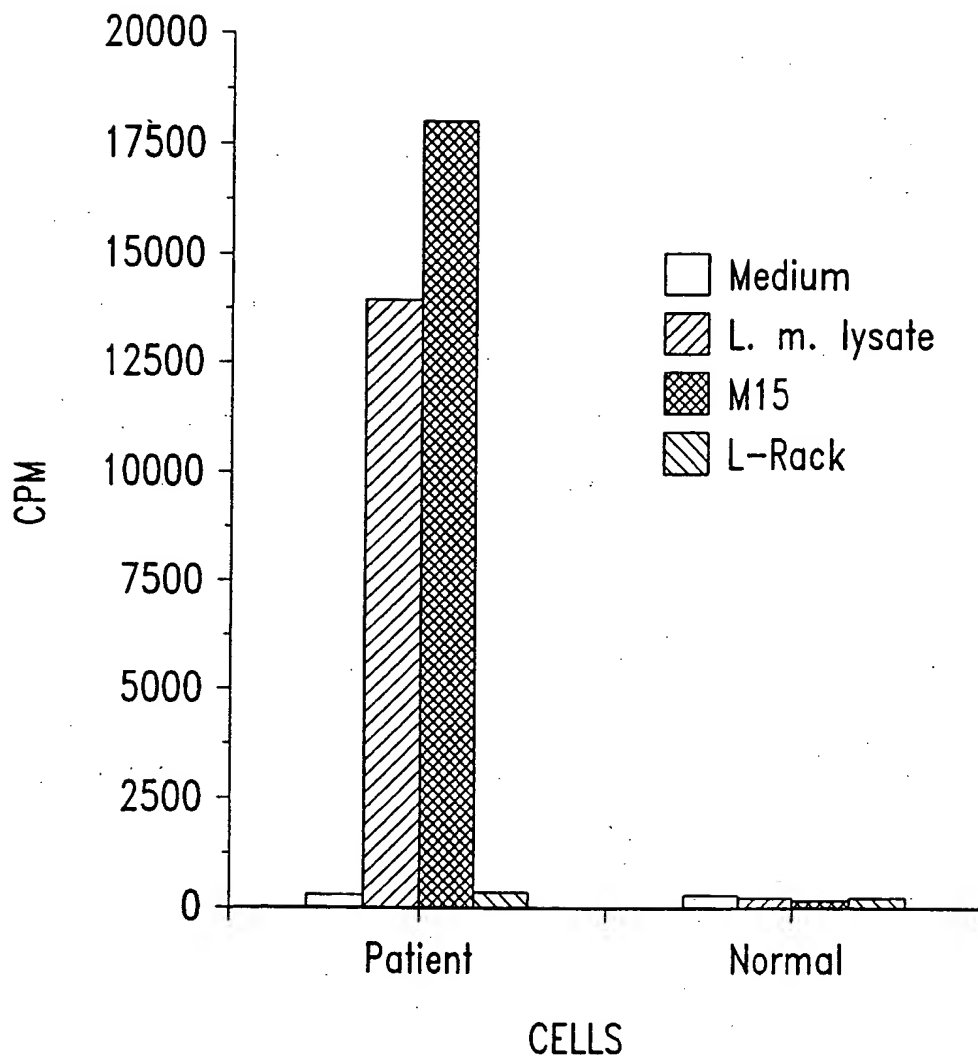
15/29

*Fig. 15*

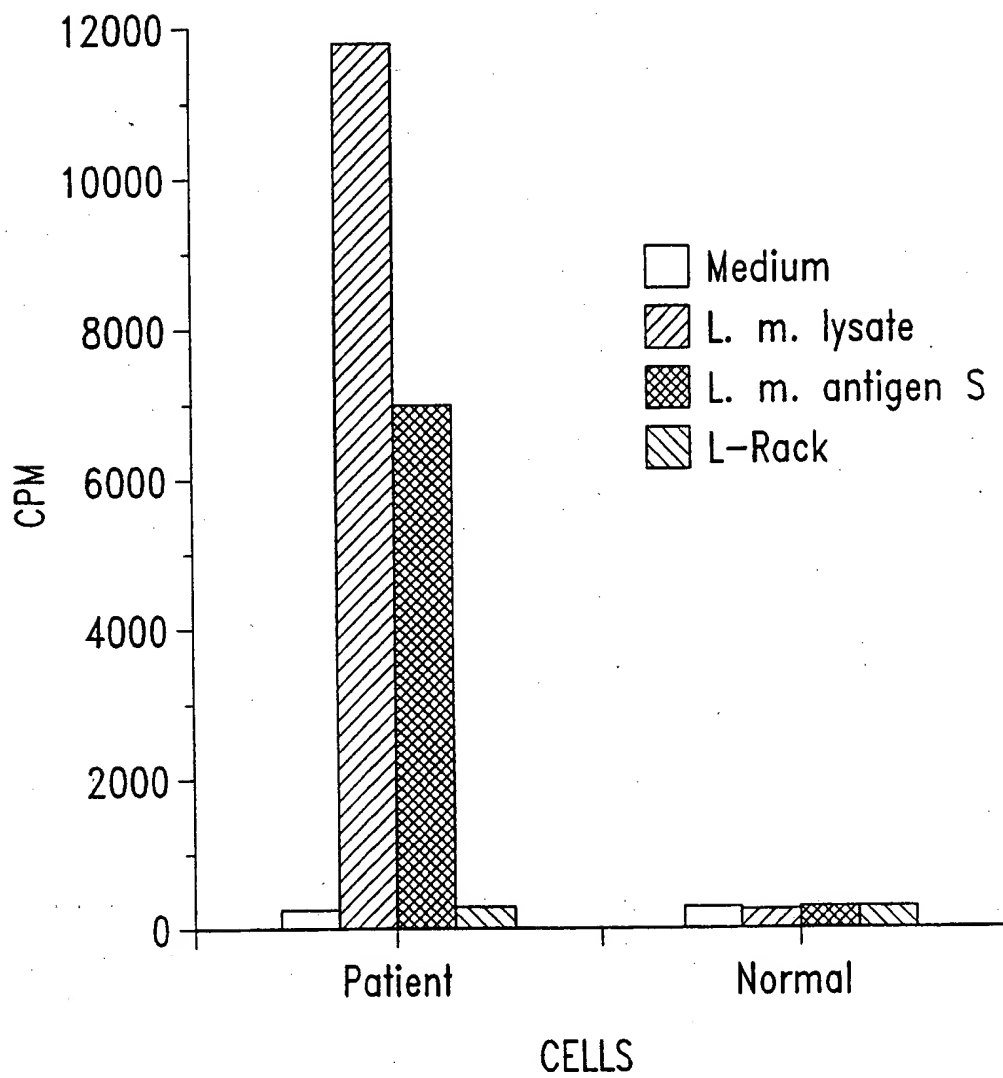
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*Fig. 16*

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*Fig. 17*

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*Fig. 18*

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-----SLTDPAVLGEETHLRVVRVPDKANKTLTVEDNGIGMTK 85
 MTETFAFQAEINQLMSLIINTFYSNKEIFLRDVISNASDACDKIRYQ.....DA. R. C.....E..... 85
EL.....NQ.....D. S. I.....T..... 85
 MPEETQTQDQPMEEEV.....A.....EL.....S. L.....E.....SK. DSGKE. HINLI. N. QDRA. IV. T..... 100

P P
 ADLVNLTGTIARSGTKAFMEALEAGGDMSIGQFVGIFYSAYLVADRVTVVSKNNSDEAY-VESSAGGTFTITSVQESDMKRGTTTLHLKEDQOEYLEE 184
A.....T.....V. V.....AP.....LPARI.....L.....A 185
 E.....D.....T.....V. PTPDC. L.....RIV..... 185
 I.....K.....Q. A. I.....EK.....IT. H. D. Q. A.....S. VRTDTGEP. G. KVI.....T..... 200

RRVKELIKKHSEFIGYDIELMVEKTAKEVTDE----DEEDESKKKSCGDEGEPKVEEVTGG-ED-KKKKTKKVKVKKT-YEVK---NKKPLWTRD 274
 L.....T.....---A. ADE. GE.....E.....T. E---Q--- 272
 L. D.....AT.....D. --AAATKNEEGE.....KDDAE. GE.....TQE-FV. Q--- 275
 I. IV. Q. P. T. F. ERD. S. DEAEK. DKE. E. E. E. E. SEDKPEI. DVGSD. E. DGD. K. KKI. EK. ID. EEL. T. I. N 300

→ Lbhsp83b

TKDVTKEEYAAFYKAISNDWEDTAATKHFVSEGQLEFRATFVVKRAPFDMFEPNKKRNKLYVRRVFIMDNCECLCPDWLGFVKGVDSEDLPLNISR 374
 P.....PP.....M.....L..... 372
 P.....EPLS.....L.....S.....E. A. R..... 375
 PD. I. N. GE. SLT. HL. V. LL. R. L. NR. K. E. I. EY. N. IR. 400

ENLQONKILKVIKKNIVKKCLELFEEIAENKEDYQFYEQFGKNIKLGIHEDTANRKKLMELLRFYSTESGEEMTLKDYVTRMKPEQKSIYYITGDSKK 474
 M. V.....V.....A. N..... 472
 A.....K.....V.....S.....H. S. D.....EG. C. V..... 475
 M. S. L. T. L. D. N. K. S. SQ. S. Y. TSA. D. VS. C. EN. H. ET. D 500

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 T. Q. R. F. I.....T.....E. T. Y. R. A. D.....V.....A..... 575
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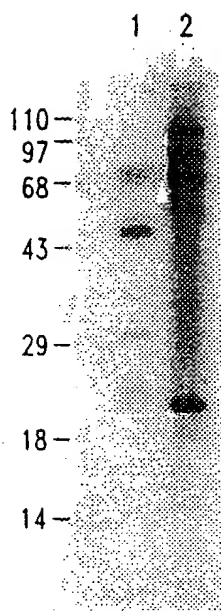
P

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 M.....M.....L. K.....E..... 671
 SA. M. I. A. V. K.....Y.....A.....T.....S.....H.....D 675
 TY. T. N. R. KA. N. TMG. AA. HL. I. S. ET. QKA. K. S. I. YE. A. S. S. E. QTH. N. Y. GI. 700

EE--EEVA-EAPPAEAPAEVTAGTSSMEQVD 703 Lbhsp83
 E--E. V. AV. T.....L..... 701 Lahsp83
 D--NGNE. E. A. V. PV..... 704 Tchsp83
 DDPTADDTSA. VTE. MP. L. GDD. R. E. 734 Huhsp89

Fig. 19

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*Fig. 20*

GAATTCGGCAGCAGGTTTCTGTACTTTATTGCTTCCAGCCCTTATTCACTCTTCGATTTCCTCTAACACCATGTCCCTCCGAGCCACCTTTATTGCCGTC

100

5'-Adaptor | Splicad-leader | 5'-UT

M S S E R T F I A V

AAGCCGGACGGCGTGCAGCGCGGCCTCGTTGGCGAGATCATCGCCCGCTTCGAGCGCAAGGGCTACAAGCTCGTCGCCCTGAAGATACTGCAGCCGACGA

200

K P D G V Q R G L V G E I I A R F E R K G Y K L V A L K I L Q P T

CGACGAGCCAGGGTCACTATAAGGACCTTTGCTCCAAGCCGTTTTTCCCGGCCCTTGTGAAGTACTTCTCCTCTGGCCCGATCGTGTGTATGGTGTG

300

I E D A D G H Y K D L C S K P F F P A L V K Y F S S G P I V C M V W

CGAGGTAAACCTGGTGAAGAGCGCGCGCTGCTGCTCGGCGGACGAACCCGGCGACTCACAGCCCGGCACGATCCGTGGCGACTTTGCCGTGGAT

400

E G K N V V K S G R V L L G A T N P A D S Q P G T I R G D F A V D

GTGGCCCGCAACGTGTGCCACGGGTCGACTCTGTGGAGAGCGCGGAGCGGAGATCGCCTTTTGGTTCAAGGCGGATGAGATCGCGAGCTGGACGTCGG

500

V G R N V C H G S D S V E S A E R E I A F W F K A D E I A S W T S

ACTCCGTGTCCAGATCTATGAGTAACGGTGATTGCGGACACGCTTTGAGGACGTAGCTGTACCCCAATGAATTCTTCTCTGAAAACCATCATAAGC

600

H S V S Q I Y E

CTCTTAAGAGGTTATTTTCTTGATCGATGCCCGGTGGTGACCAGCACCATTCCCTTTATCGGATCACTCACACTCCTAGCGAATCATGTAGTCCGGTGA

700

3'-UT

GAGTGGGCTCTGGAGGAGACTGTTGTGTAGCCATGGCTTCAGGAGAGAAAACAAAATACAAGGAAAGGCAATATGTAACATATGGGGTTCCTTTTTTACT

800

3'-UT

ATGCAAAGTTTTTATACTCCTGATCGGCAAAAACAACAACCGCCATACACCAAGAGCAAATGCTTTCTCTCGGGACTGTGCTTCTGTTTTTTTTT

900

3'-UT

ATGAAGGAGTGACTCGCGCGATGAAAAGTGTGTGCGTGGGAGATGTATTTCTTTTTTTGTTTCATAGTGGCGACAGCTCACTGTTGACGATGACAAAAA

1000

3'-UT

AAAAAAAAAAAACTCGAG 1019
Poly A tail/ Xhoi

Fig. 21

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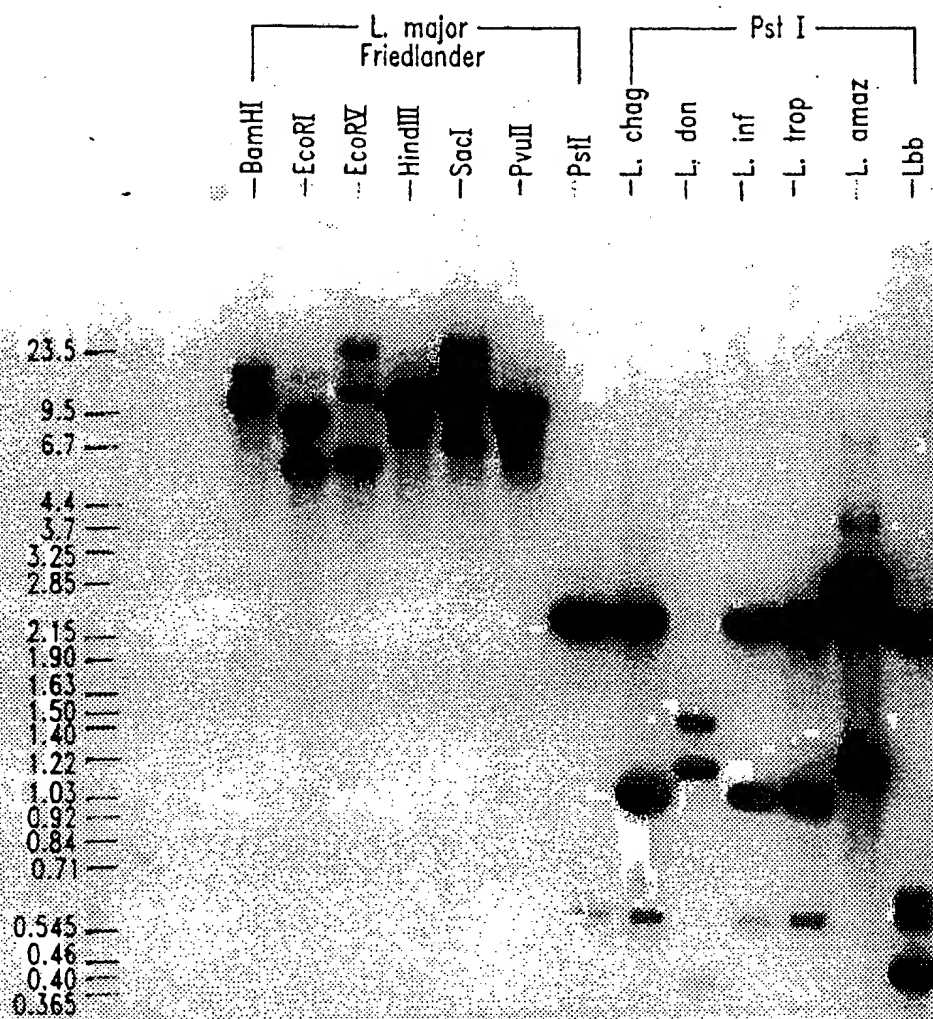
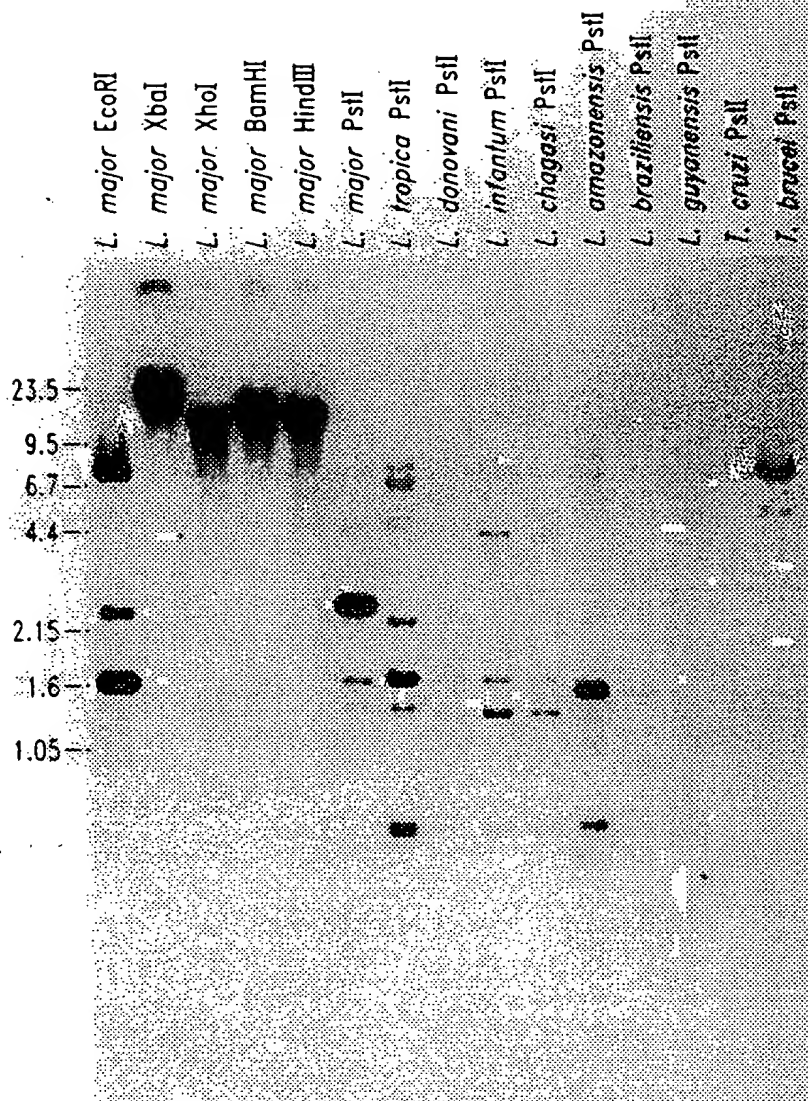


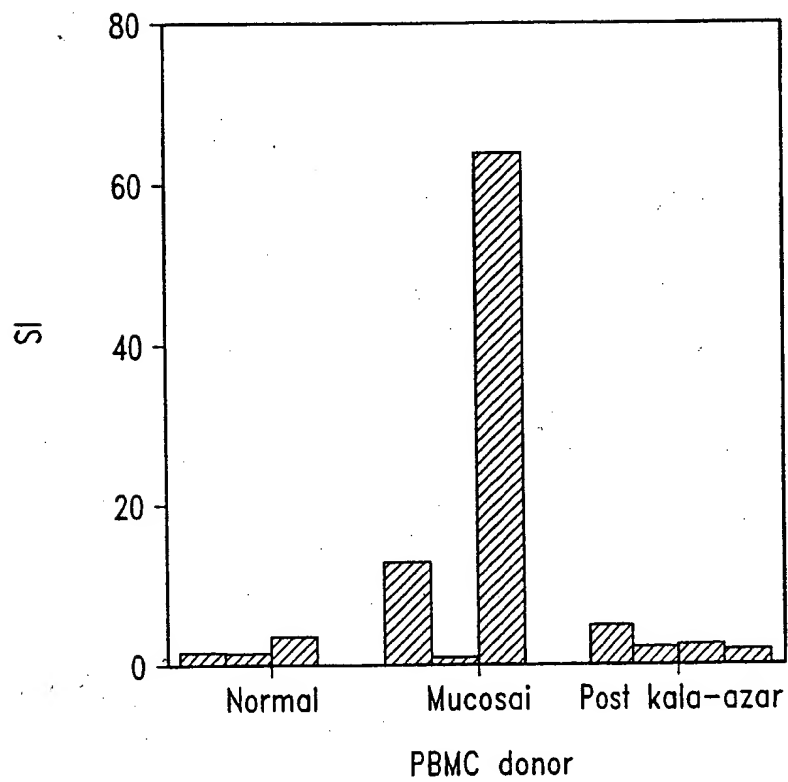
Fig. 22

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*Fig. 23*

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Proliferative response of human PBMC to MAPS

*Fig. 24*

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Proliferative Responses of *L. major*-infected BALB
(20 days post-infection) to recombinant MAPS protein

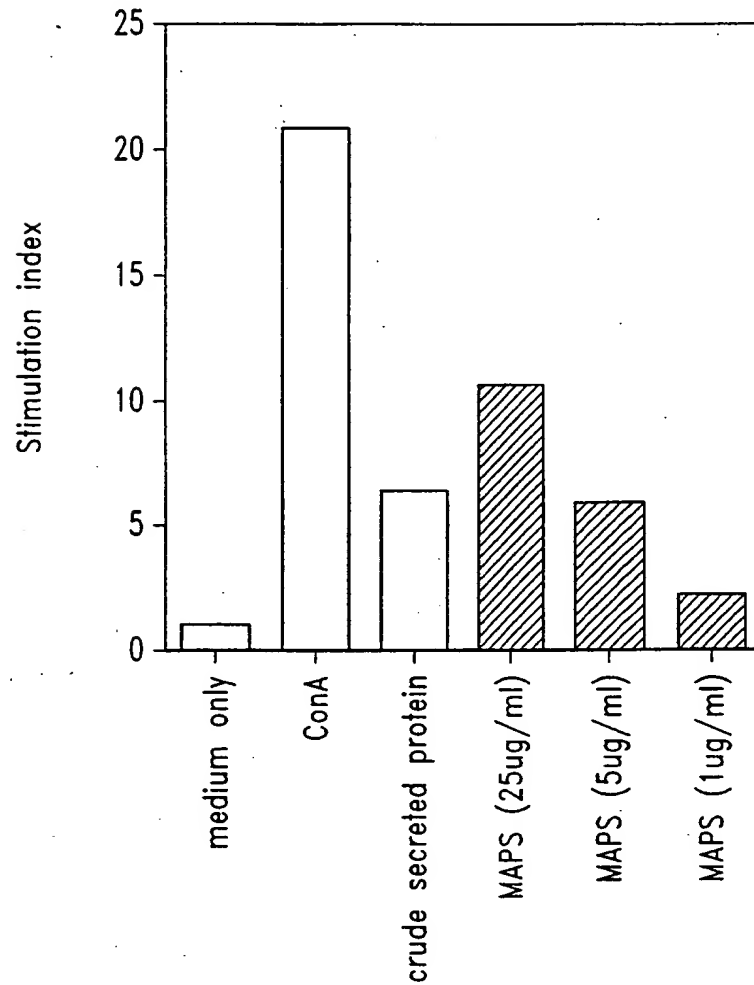


Fig. 25

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ELISA analysis of MAPS-specific antibody titre
human leishmaniasis patient sera (8/27/96)

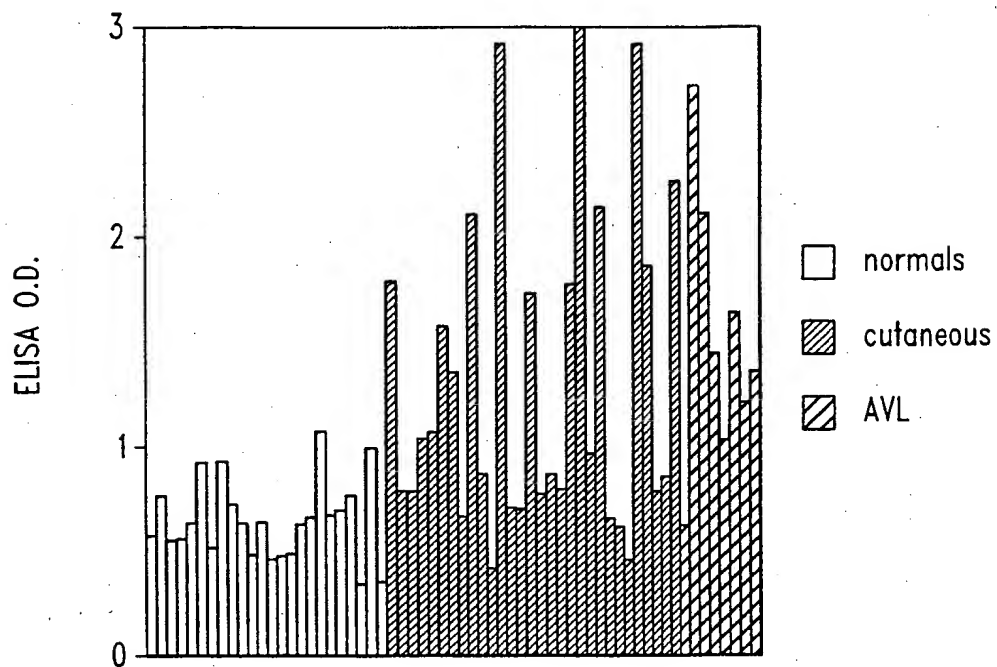


Fig. 26

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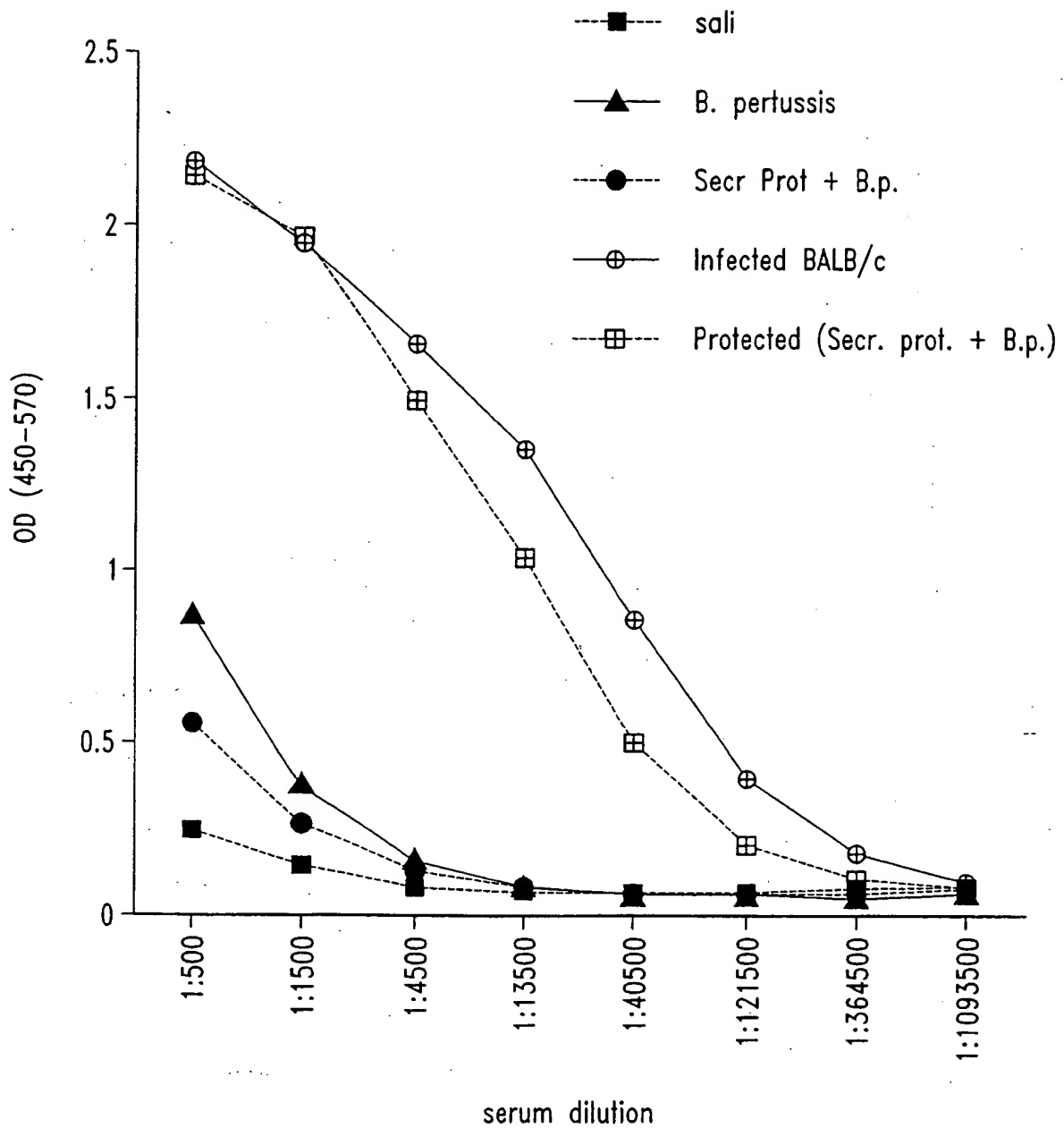
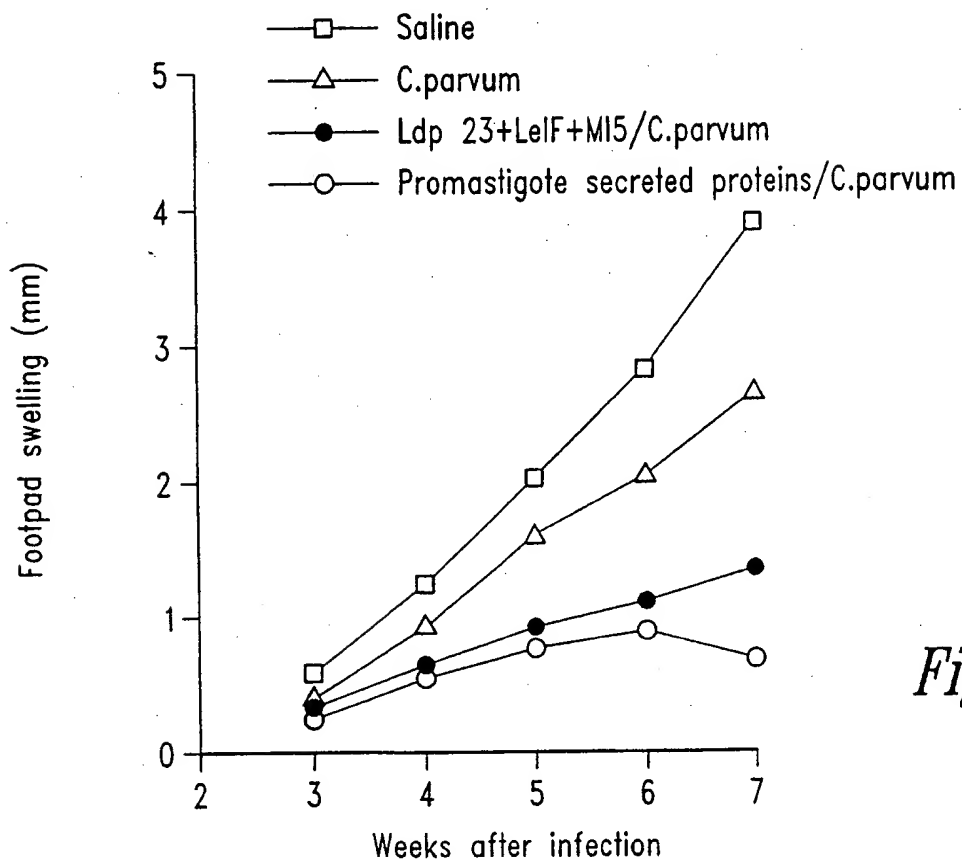
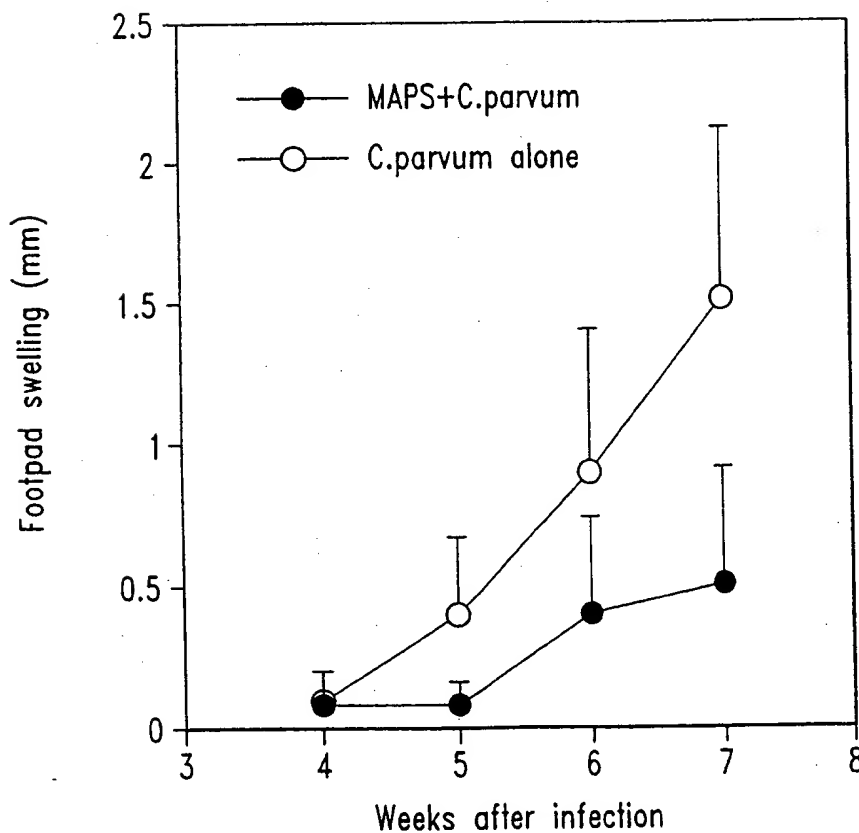
Antibody Response of Immunized/Ir
BALB/c Mice to MAPS

Fig. 27

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Protection against infection with *L. major* in BALB/c mice immunized
leishmanial antigens.

*Fig. 28**Fig. 29*

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Proliferation rLcgSP Antigens

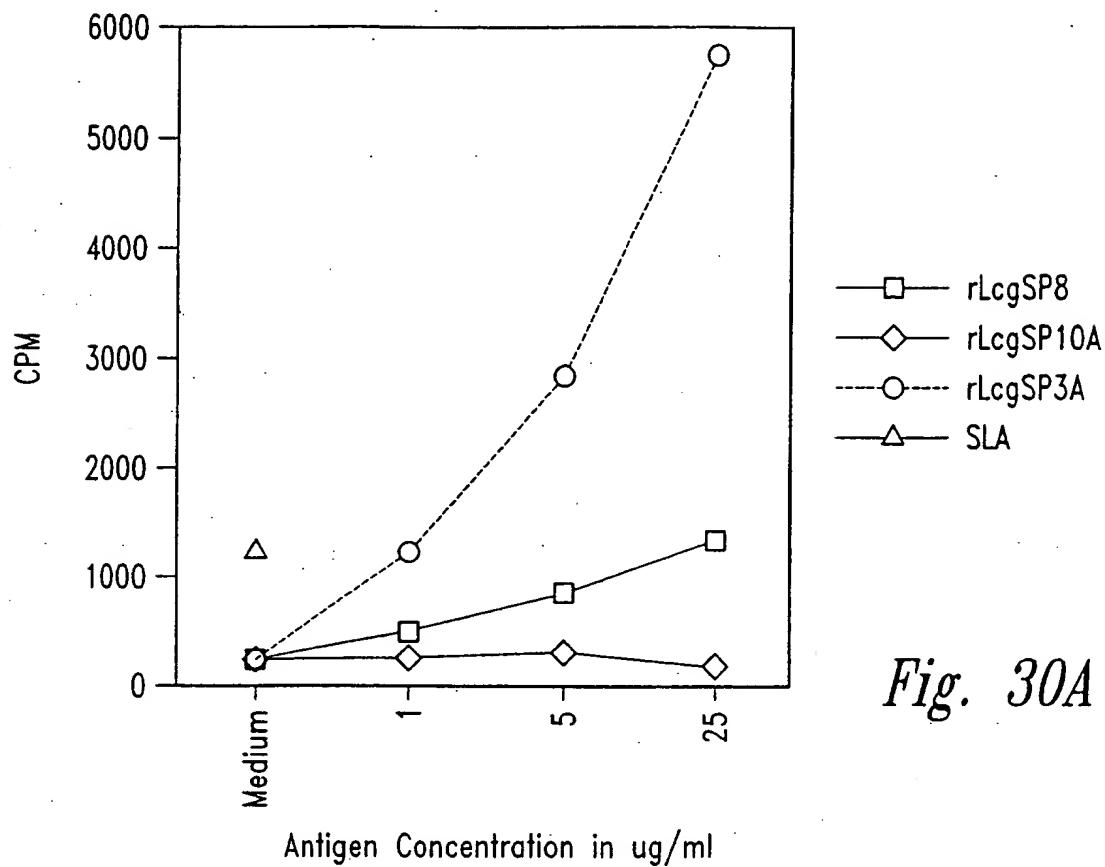


Fig. 30A

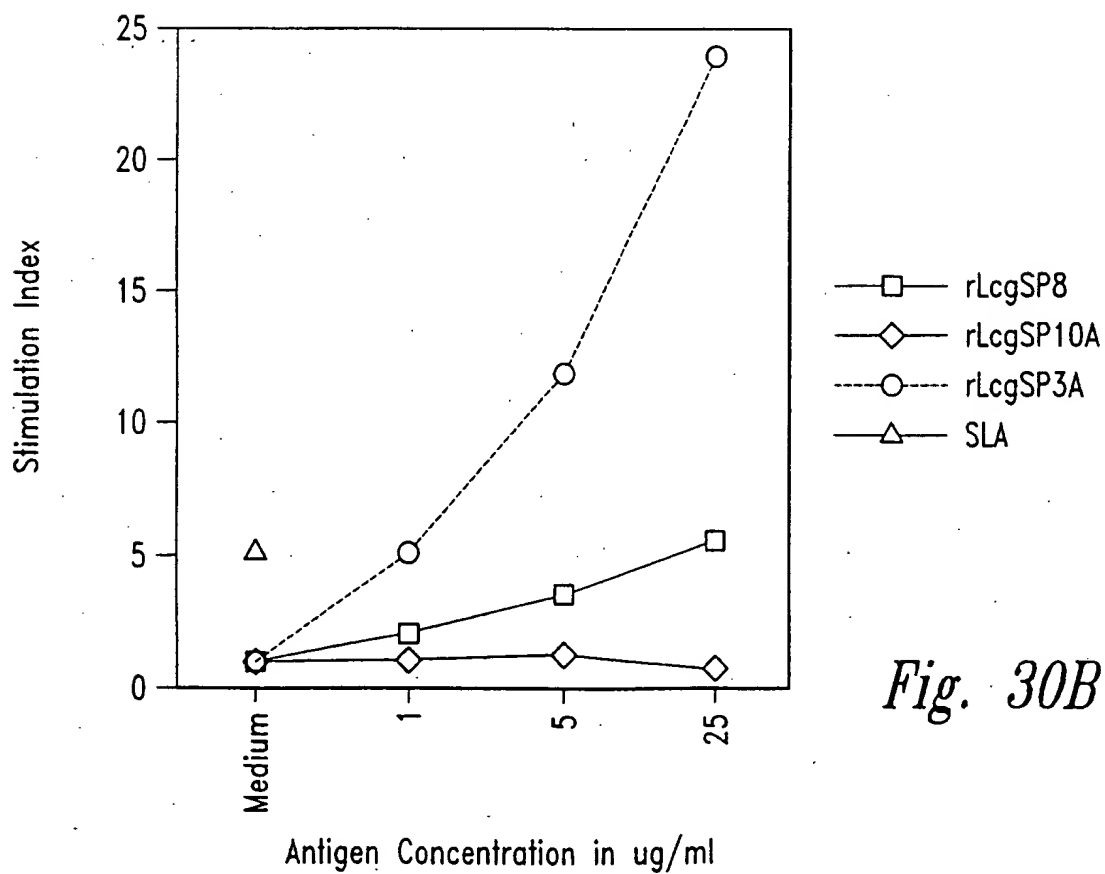


Fig. 30B

INTERNATIONAL SEARCH REPORT

Internati Application No
PCT/US 98/03002

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/30 C07K14/44 C12N15/63 C12N5/10 A61K39/008
A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 95 29239 A (CORIXA CORPORATION) 2 November 1995 see page 2, line 17 - page 3, line 37 see page 5, line 8 - page 23, line 9; examples --- -/--	1-6, 25, 27-29, 31, 35-40, 42-55



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

Date of the actual completion of the international search

28 May 1998

Date of mailing of the international search report

27. 08. 1998

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

MONTERO LOPEZ B.

INTERNATIONAL SEARCH REPORT

Internal I Application No

PCT/US 98/03002

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	<p>WO 97 11180 A (CORIXA CORPORATION) 27 March 1997</p> <p>see page 2, line 20 - page 3, line 37 see page 6, line 16 - page 7, line 20 see page 9, line 4 - line 19 see page 12, line 20 - page 14, line 17 see page 15, line 3 - page 16, line 26 see page 17, line 8 - line 30; claims; examples 1,7,8</p> <p style="text-align: center;">-----</p>	<p>1-6,25, 27-29, 31, 35-40, 42-55</p>

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 98/03002

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see continuation sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

see continuation sheet, subject 1

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-6, and partially 25, 27-29, 31, 33, 35-40, 42-55

Polypeptide comprising an immunogenic portion of a Leishmania antigen having SEQ ID NO:2, variant thereof, DNA molecule encoding it, expression vector and host cell comprising the same, pharmaceutical compositions and vaccines comprising the polypeptide, vaccine comprising the DNA molecule, diagnostic kit

2. Claims: 7-12, and partially 25, 27-29, 31, 33, 35-40, 42-55

Polypeptide comprising an immunogenic portion of a Leishmania antigen having SEQ ID NO:4, variant thereof, DNA molecule encoding it, expression vector and host cell comprising the same, pharmaceutical compositions and vaccines comprising the polypeptide, vaccine comprising the DNA molecule, diagnostic kit

3. Claims: Partially 13, 16-19, 25, 27-31, 33-40, 42-55

Polypeptide comprising an immunogenic portion of a Leishmania antigen comprising SEQ ID NO:22, variant thereof, DNA molecule encoding it, expression vector and host cell comprising the same, pharmaceutical compositions and vaccines comprising the polypeptide, vaccine comprising the DNA molecule, diagnostic kit

4. Claims: Partially 13, 16-19, 25, 27-31, 33-40, 42-55

Idem as subject 3, for SEQ ID NO:24

5. Claims: Partially 13, 16-19, 25, 27-31, 33-40, 42-55

Idem as subject 3, for SEQ ID NO:26

6. Claims: Partially 13, 16-19, 25, 27-31, 33-40, 42-55

Idem as subject 3, for SEQ ID NO:36

7. Claims: Partially 13, 16-19, 25, 27-31, 33-40, 42-55

Idem as subject 3, for SEQ ID NO:37

8. Claims: Partially 13, 16-19, 25, 27-31, 33-40, 42-55

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Idem as subject 3, for SEQ ID NO:38

9. Claims: Partially 13, 16-19, 25, 27-31, 33-40, 42-55

Idem as subject 3, for SEQ ID NO:41

10. Claims: Partially 13, 16-19, 25, 27-31, 33-40, 42-55

Idem as subject 3, for SEQ ID NO:49

11. Claims: Partially 13, 16-19, 25, 27-31, 33-40, 42-55

Idem as subject 3, for SEQ ID NO:50

12. Claims: Partially 13, 16-19, 25, 27-31, 33-40, 42-55

Idem as subject 3, for SEQ ID NO:51

13. Claims: Partially 13, 16-19, 25, 27-31, 33-40, 42-55

Idem as subject 3, for SEQ ID NO:52

14. Claims: Partially 13, 16-19, 25, 27-31, 33-40, 42-55

Idem as subject 3, for SEQ ID NO:53

15. Claims: Partially 13, 16-19, 25, 27-29, 31, 33, 35-40,
42-55

Idem as subject 3, for SEQ ID NO:82

16. Claims: 14, 15, and partially 16, 25, 27-29, 31, 33, 35-40,
42-55

Antigenic epitope of Leishmania antigen comprising SEQ ID NO:43, polypeptide comprising at least two such contiguous epitopes, DNA molecule encoding it, pharmaceutical compositions and vaccines comprising the polypeptide, vaccine comprising the DNA molecule, diagnostic kit

17. Claims: 20-24, 26, 32, 41 and partially 27-30, 33-39, 42-55

Polypeptide comprising an immunogenic portion of a

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Leishmania antigen comprising SEQ ID NO:20, variant thereof,
DNA molecule encoding it, expression vector and host cell
comprising the same, pharmaceutical compositions and
vaccines comprising the polypeptide, vaccine comprising the
DNA molecule, diagnostic kit

18. Claims: Partially 56-62

Pharmaceutical compositions and vaccines comprising a
polypeptide comprising an immunogenic portion of a
Leishmania antigen comprising SEQ ID NO:39 or a variant
thereof

19. Claims: Partially 56-62

Idem as subject 18, for SEQ ID NO:42

20. Claims: Partially 56-62

Idem as subject 18, for SEQ ID NO:55

21. Claims: Partially 56-62

Idem as subject 18, for SEQ ID NO:61

22. Claims: Partially 56-62

Idem as subject 18, for SEQ ID NO:62

23. Claims: Partially 56-62

Idem as subject 18, for SEQ ID NO:80

24. Claims: Partially 56-62

Idem as subject 18, for SEQ ID NO:81

25. Claims: Partially 56-62

Idem as subject 18, for SEQ ID NO:83

26. Claims: Partially 56-62

Idem as subject 18, for SEQ ID NO:84

27. Claims: Partially 56-62

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Idem as subject 18, for SEQ ID NO:85

28. Claims: Partially 56-62

Idem as subject 18, for SEQ ID NO:86

29. Claims: Partially 56-62

Idem as subject 18, for SEQ ID NO:87

INTERNATIONAL SEARCH REPORT

Information on patent family members

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